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OHIO RIVER POLLUTION SURVEY: ORGANIZATION AND METHODS OF LABORATORY STUDIES

Public Health Service
Cincinnati, Ohio

1942

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ORGANIZATION AND METHODS OF LABORATORY STUDIES

Supplement "B" to
Final Report to the Ohio River Committee
Ohio River Pollution Survey

1798

FEDERAL SECURITY AGENCY
U. S. PUBLIC HEALTH SERVICE
STREAM POLLUTION INVESTIGATIONS STATION
CINCINNATI, OHIO
1942

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ORGANIZATION AND METHODS OF LABORATORY STUDIES

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ORGANIZATION AND METHODS OF LABORATORY STUDIES

The laboratory studies carried out in connection with the Ohio River Pollution Survey have constituted one of the three major parts of the Survey, the other two being concerned, respectively, with the collection of data on sources of pollution and with the measurement of the volume of flow of the streams in the Ohio River Basin during the period of the Survey. From the standpoint of operations, the laboratory studies have been in some respects the most difficult and complex activity connected with the Survey, as they have involved the systematic examination of practically every stream in the 204,000 square miles of territory included within the Basin, except in certain limited areas of the Scioto,* Tennessee, Cumberland and Miami watersheds, where recent laboratory observations had been carried out by other agencies.

The methods and results of the routine laboratory operations of the Survey have been described somewhat briefly in connection with the main report of the Survey, including a presentation of the results of the stream examinations in complete tabular form. Owing to the limited space available in the main report, however, it has not been practicable to give in that report certain details concerning the planning and methods of carrying out the laboratory operations of which a record would be desirable elsewhere for the benefit of those undertaking similar work in the future.

(*) See Public Health Bull. 276, U. S. Public Health Service.

Objectives of Laboratory Studies

The main objectives of the laboratory studies have been as follows:

1. To ascertain, by means of systematic laboratory tests, the sanitary quality of the waters of the main Ohio River and its tributary streams at various points throughout the entire drainage basin and especially above and below recognized sources of pollution.

2. To examine streams in the mining sections of the Basin for evidences of acid mine wastes and their effects.

3. To study, so far as practicable, certain special problems resulting from stream pollution along the Ohio River and its tributary streams, notably:

    (a) The measurable effects of mine sealing on the acidity of streams receiving mine wastes.

    (b) The presence in stream waters of substances causing tastes and odors in water supplies, notably phenolic substances causing chlorophenol tastes, which have been most commonly prevalent in water supplies of the Ohio Basin.

    (c) The presence of sludge deposits in pooled sections of the main Ohio River.

4. To observe, by methods of biological study, the effects of sewage and industrial wastes pollution on the plankton and higher aquatic life, notably fish life, in various typical streams throughout the Ohio Basin.

In undertaking a systematic examination of stream waters throughout the Ohio River Basin, it was essential to have in mind the divergent effects which variations in the flow and temperature of a stream may have on the sanitary quality of its water at different times. From the standpoint of general sanitary conditions, the more critical flows and temperatures usually occur during the dry-weather conditions of summer, when the volumes of diluting water carried by streams are at their minimum and increased water temperatures tend to promote the more rapid bio-chemical decomposition of organic polluting materials at a time when the normal dissolved oxygen content of a stream is at its lowest level. From certain other standpoints and notably with respect to the use of streams for water supplies, more critical conditions may occur during periods of increased stream flows, especially following prolonged low water conditions.

Although every effort was made to observe stream conditions over as much of the Basin as practicable during "critical" flow periods such as above indicated, it was not feasible to carry out such observations simultaneously except over restricted areas, because each laboratory unit was limited in its coverage to a radius of about 50 miles from a particular location point. Moreover, the long periods of drought which occurred throughout the years 1939 and 1940, though facilitating the extension of laboratory observations to more areas during low-water periods than otherwise would have been possible, reduced correspondingly the opportunities for high water observations during periods of the winter and spring when these conditions normally would be expected to prevail. One advantageous circumstance which resulted from the abnormal prolongation of drought conditions through the winters of 1939 and 1940 was the opportunity thus provided for observing some streams under low water conditions both in summer and in winter, with stream temperature the only important variable to be considered as affecting the sanitary quality of the streams during these two different seasons.

The effects of mine wastes on streams in large sections of the Ohio River Basin devoted to soft coal mining are known in general terms, but had not been measured systematically by means of stream observations to any considerable extent prior to the present Survey. Owing to the extensive program of mine sealing which has been in progress in different parts of the Basin, it was considered especially important to carry out analyses of stream waters in the areas both affected and unaffected by sealing operations. As these tests were made in connection with other laboratory operations, their results have had a definite significance, both in themselves and in relation to those of routine examinations bearing on general sanitary conditions in the same streams.

The special studies above enumerated have been carried out for different reasons. The study of changes in stream acidity resulting from mine sealing were instituted in order to ascertain more definitely than could be determined by ordinary observational methods the effects of mine sealing in quantitative terms of stream improvement. This study, instituted late in 1940, was confined to a test area near Morgantown, W. Va., where the effects of complete and partial mine sealing, with an unsealed area as a control, could be observed over a period of several months with all other conditions, geological and meteorological, practically the same for each subdivision of the test area.

The study of substances causing tastes and odors in water supplies was undertaken in cooperation with the departments of health of Ohio, Pennsylvania and West Virginia, as presenting one of the more serious problems of industrial wastes pollution affecting water supplies in the upper section of the Ohio Basin. This study was centered in the lower Kanawha River and in the Mahoning-Beaver area.

The study of mud deposits was one of special interest as bearing on the extent and distribution of these deposits in pooled sections of the river and on the degree to which they are affected by organic solids originating in sewage and industrial wastes. The deoxygenating effect of sewage deposits, where prevalent in relatively large quantities, would tend to impose an added burden on the oxygen resources of the overlying stream.

The biological study of the effects of stream pollution on plankton and higher aquatic life was an essential part of the regular laboratory survey, as it dealt with an important phase of the problem untouched by the other laboratory observations. Probably the most important practical element in the biological phase of this problem has been the effects of pollution on fish life. This question, though studied by the U. S. Bureau of Fisheries in connection with its other investigations, had not been considered previously with specific reference to the pollution of the Ohio River and its tributary streams. It had not been touched upon in any previous sanitary surveys of the Ohio River. Its importance in relation to the recreational use of streams in the Ohio Basin, as well as to the maintenance of desirable sanitary conditions in streams devoted to other uses, afforded ample justification for a thorough study of it in connection with this survey. As this study has been covered fully in a separate report by the Biologist of the Survey, it will not be dealt with in the present supplement.

Organization of Laboratory Operations

In accordance with a general plan of operations adopted in October, 1938, the Stream Pollution Investigations Station of the Public Health Service at Cincinnati was designated to undertake the necessary laboratory work incidental to the Ohio River Pollution Survey. The instructions issued in this connection included authorization for detailing necessary personnel to the work from the regular staff of the Station, for nominating additional personnel, and for obtaining all supplies and equipment necessary to carrying out the laboratory operations. An officer of the Cincinnati Station was detailed to organize and direct the laboratory work and other officers have acted as technical advisors from time to time.

The original plan of the Survey, as approved by the supervisory Ohio River Committee, provided for a three-year period of laboratory observations, covering approximately one-third of the entire basin each year and using the same personnel and equipment from year to year. The section to be covered during the first year, 1939, included the entire area draining into the Ohio River from the Kanawha to the Kentucky River, inclusive.

In order to carry out the laboratory operations, a staff of 30 technicians, office workers and field assistants was assembled early in 1939, trained at the Cincinnati Station and assigned to duties as rapidly as thorough training permitted. Meanwhile, equipment was purchased for a fixed base laboratory at the Cincinnati Station, which was made the headquarters for the work, and a floating base laboratory was equipped on the quarterboat "Kiski", loaned for the purpose by the Corps of Engineers, U. S. Army. Active laboratory work was started at the Cincinnati laboratory in February, 1939, while the assembly of personnel and equipment was in progress. Two mobile trailer laboratories were purchased and equipped for observations in the more distant areas of the drainage basin, not readily accessible from the base laboratories located on the main Ohio River. Two motor boats, loaned by the U. S. Bureau of Fisheries, were overhauled and outfitted for sample collection work on the main river.

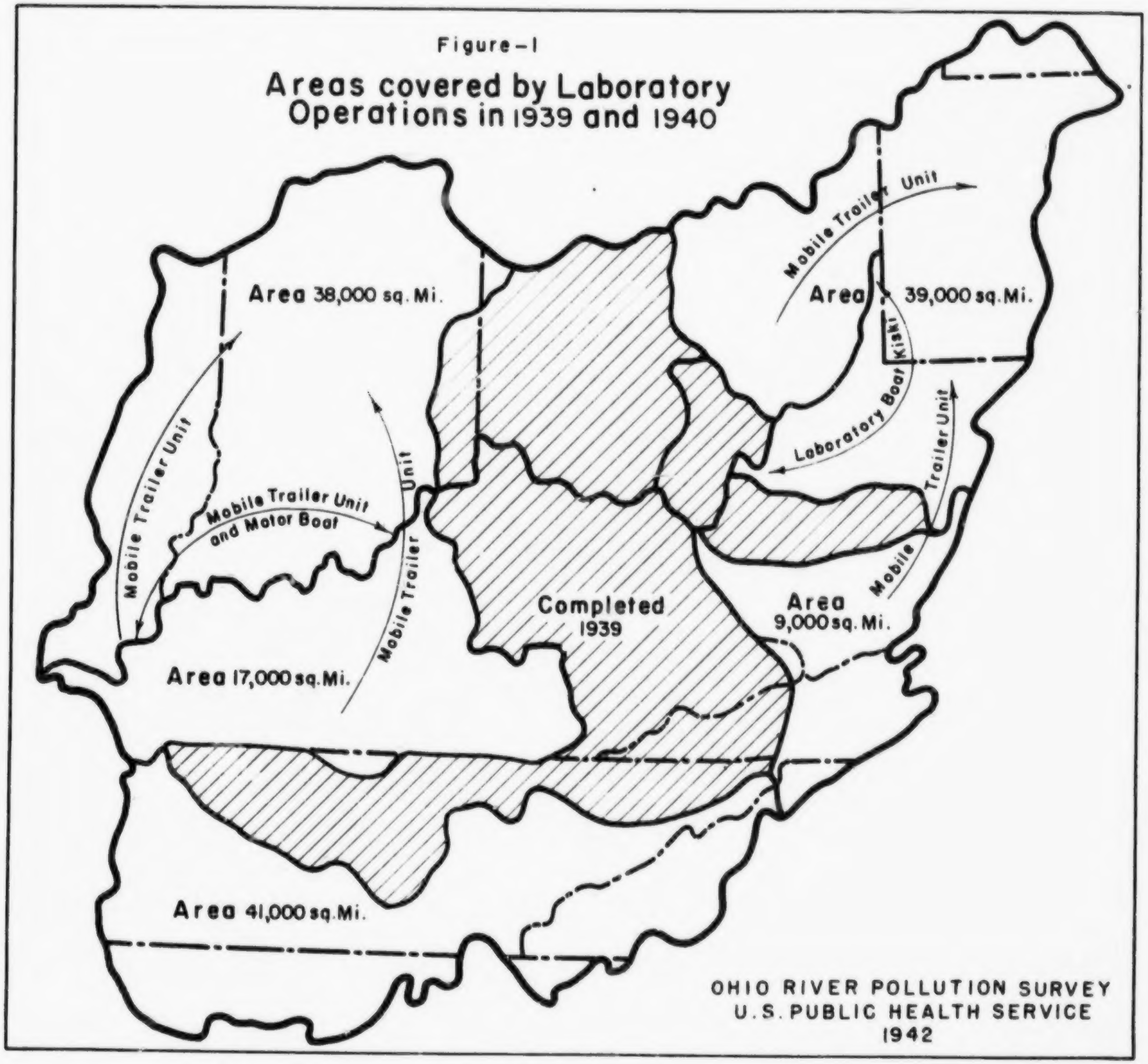
After the first year of operations had been carried out according to the original plan, the Ohio River Committee met in February, 1940, and decided to have the entire survey completed during the year 1940, so far as practicable, thus compressing into one year the work originally planned for two years. This decision involved practically a two-fold intensification of laboratory operations, with added equipment and augmented personnel. The latter consisted of twenty-seven more workers added to the original staff, making a total personnel numbering fifty-seven. Additional equipment purchased in order to carry out this intensified plan included four more mobile laboratories and two new motorboats, together with a considerable amount of laboratory apparatus and supplies.

In the accompanying map (Figure 1) is shown a double-hatched area marking the territory covered by laboratory operations in 1939, aggregating some 60,000 square miles. The unhatched portion, totaling about 144,000 square miles, was the area covered by operations in 1940, including portions of the Tennessee and Cumberland Basins surveyed by the Tennessee Valley Authority and the Tennessee Department of Health respectively. This portion of the map also shows by arrows the general routes of mobile laboratory operations and the section covered by the Kiski laboratory.

In Table 1 is shown a time schedule, by months, of the routine laboratory operations carried out during the entire period of the survey. The operations during the year 1939, in the middle-third area of the Basin were

Figure-1

Areas covered by Laboratory Operations in 1939 and 1940


Area 38,000 sq. Mi.
Area 39,000 sq. Mi.
Mobile Trailer Unit
Mobile Trailer Unit
Mobile Trailer Unit and Motor Boat
Mobile Trailer Unit
Kiski
Laboratory Boat
Mobile Trailer Unit
Completed 1939
Area 9,000 sq. Mi.
Area 17,000 sq. Mi.
Area 41,000 sq. Mi.

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shared by five different types of laboratory units, including those of the Scioto River Investigation, a separate project of the Public Health Service, and the Dayton Sewage Treatment Plant, which cooperated with the Survey in laboratory observations in the lower section of the Miami River. During this period, continuing observations by the Cincinnati and Kiski laboratories covered parts of eight tributary areas, in addition to the middle-third section of the main Ohio River from Point Pleasant to Dam 39. During the year 1940 and up to the end of March, 1941, the laboratory operations were carried out by the Kiski laboratory, along the upper section of the Ohio River, and by the six mobile trailer laboratories, in the outlying tributary areas and along the lower section of the main river. Operations in the upper and lower tributary areas in 1940 were not continuous during periods of several months, as was the case in 1939 for the middle-third area. This was due to the necessity of covering more areas by means of mobile laboratory units, which in some instances were obliged to work back and forth from one tributary area to another, in order to economize on time and travel distances.

Methods of Laboratory Operations

During the year 1939, laboratory operations along the middle third of the Ohio River were divided between the base laboratory at Cincinnati and the floating "Kiski" laboratory located at Dam 29, just above Ashland, Kentucky. During the year 1940, the upper section of the main Ohio River was covered from the Kiski laboratory at two locations, East Liverpool and Marietta, respectively. The lower section, extending from the Kentucky River to the mouth of the Ohio at Cairo, was covered by a mobile trailer laboratory with a motorboat for sample collecting in the main river. The other five trailer laboratories covered the entire outlying tributary areas except those within convenient access to the base laboratories located along the main river. Portions of the Cumberland and Tennessee river basins were omitted from the survey because they were being or recently had been covered by the Tennessee Health Department and the Tennessee Valley Authority, respectively.

TABLE 1 SCHEDULE OF ROUTINE LABORATORY OPERATIONS, BY MONTHS, 1939-41.

Watershed	1939												1940												1941			
	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A
Allegheny																			T	T	T	T	T	T				
Monongahela																	T	T	T	T	T	T	T	T				
Beaver																		T	T	T		K	K	K	T			
Muskingum																T	KT	KT	KT	K	K				K	K	K	
Little Kanawha																	K	K	K	K	K				K	K	K	
Hocking										T	T					T	T	K	K	K	K				K			
Kanawha								K	K	K	K	KT	KT	KT	KT	KT	T	T								T		
Guyandot						K	K	K	K	KT	KT	K	K	K	KT	KT												
Big Sandy						K	K	K	K	K	KT	KT	K	K	K	K												
Scioto	S	S	S	S	S	S	SC	C	TC	TC	TC	C	C	C	C	C												
Little Miami	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C											
Licking		C	C	C	C	C	C	C	CT	CT	CT	CT	C	C	C													
Miami		C	C	C	C	CD	CD	CDT	CDT	CD	CD	CD	C	C	C	C												
Kentucky			C	C	C	C	C	C	CT	CT	CT	C		C	C	C			T			T			T			
Salt																			T	T		T				T		
Green																				T	T	T	T			T		
Wabash																			T	T	T	T	T			T		
Cumberland																				T	T	T	T		T	T	T	
Tennessee																					T	T	T		T	T	T	
Ohio-Pittsburgh-Dam 13																	T	T	T		T	K	K	K	K		T	
Ohio-Dam 14 to Dam 22 Incl.																	K	K	K	K	K				K	K	K	
Ohio-Pt.Pleasant-Dam 32						K	K	K	K	K	K	K	K	K	K	KT												
Ohio-Dam 33 to Dam 38 Incl.	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C											
Ohio-Below Dam 39 to Mouth																			T	T	T	T	T	T	T	T	T	

Symbols:
C - Cincinnati Laboratory   K - Kiski   T - Trailer laboratory unit   S - Scioto River Investigation   D - Dayton Sewage laboratory.

Sample Collections

Through the cooperation of the Corps of Engineers, arrangements were made for the regular collection of samples at the locks and dams along the upper and middle section of the main Ohio River. These collections were made by the lock personnel, using boats with outboard motors, in accordance with a schedule providing for two or three collections each week at each lock.* Samples were collected at three points on a cross-section located 1,000 to 1,500 feet upstream from the dam. Deep water sampling equipment was used, similar to that previously developed by the Public Health Service in other stream surveys. All samples were collected at mid-depth points in the stream cross-section. After being collected, the samples were transported by automobile without delay to the nearest base laboratory for analysis.

For the collection of samples from tributary points located within convenient travel distance from the base laboratories, regular personnel were employed by the survey. These sample collectors used their own automobiles for transportation and either highway bridges or boats as means of reaching the stream sampling points. On the tributary streams samples were collected generally at mid-points and mid-depths, using a deep or shallow water sampler, as needed. For reaching some points at the mouths of main tributaries and in the Ohio River between the locks and dams, motorboats were used, being operated by Survey personnel.

In Figures 2 to 7, inclusive, are shown illustrative views of the equipment used in collecting samples of the stream waters and bottom mud deposits.** Figure 2 gives two views of the deep water sampler, assembled and unassembled. The sampler is made of heavy cast bronze, with a rubber gasket sealed cover and weighted with lead in the bottom. In Figure 3 is a view of the sampler mounted on a bridge hoist, ready for action. Figure 4 pictures a surface or shallow stream sampler, mounted on a pole. In Figure 5 is shown a deep water mud deposit

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(*) For detailed instructions on the collection of these samples, see Appendix 1, Memorandum No. 1.

(**) For a detailed description of this equipment, see article on "Mechanical Aids for Stream Surveys," by C. T. Carnahan, Public Health Reports, Vol. 56, No. 16., Apr. 18, 1941, pp 815-821.

dredge of the clam-shell type, mounted on a hoist and operated by a hand-winch. This dredge can be operated in stiff materials such as clay, and will bring up about one-half cubic foot or more of mud. Figure 6 shows two views of a light-weight surface mud sampler, one being in an open position as lowered to the deposit and the other, in a closed position after collection of the mud sample. Both this and the clam-shell dredge were devised by Public Health Engineer C. T. Carnahan of the Public Health Service. In Figure 7 is pictured one of the motorboats at the time of collecting a sample of river water. This was one of the two older boats loaned by the U. S. Bureau of Fisheries.

In Figure 8 is an illustration of the floating laboratory Kiski loaned by the Corps of Engineers, the lower deck being used for laboratory work and the upper deck as an office and living quarters for the crew and unmarried men of the technical staff. The members of the staff quartered on the boat provided and maintained their own sleeping quarters and commissary. Every man in the staff was called upon to help with the mooring and navigation of the boat, as required, though three shipkeepers were employed especially for this purpose. A 24 hour watch was maintained by these men as a part of their regular duties. The Engineer in Charge of the laboratory also was responsible for the maintenance and navigation of the boat and its equipment.

Trailer Laboratories

The general method of operating the mobile laboratories was as follows: Before undertaking the survey of a given area, maps were prepared at the Cincinnati headquarters showing the sampling point locations for different stream areas and a central place in each area at which the mobile laboratory could be located for a period of two or three weeks while the particular area was being covered by stream examinations. Preliminary correspondence resulted in a tentative location for each stopping place of the mobile units. The travel routes for each unit were laid out for successive areas to be covered and an estimate made of the necessary time schedule of travel. As previously noted, a field engineer was sent in advance to complete detailed arrangements for each location and to survey the tentative sampling

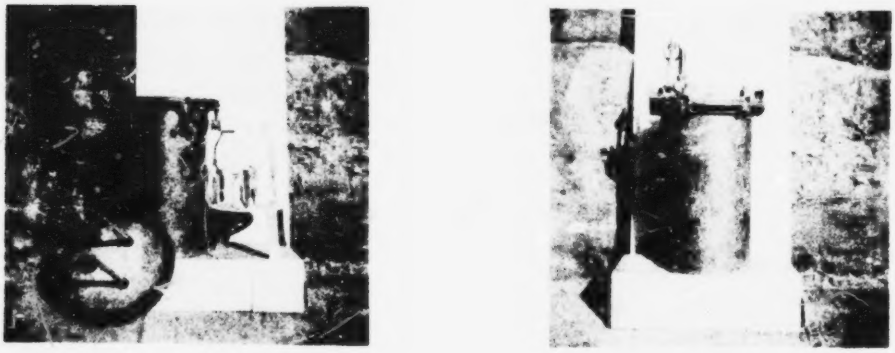


Figure 2 - Deep water sampler, open and closed.

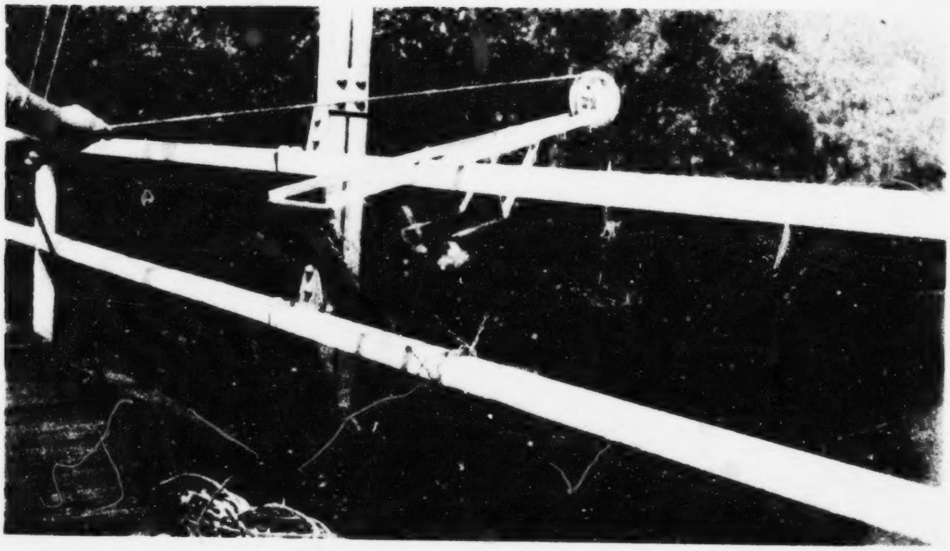


Figure 3 - Sampler hoist, mounted on a bridge.

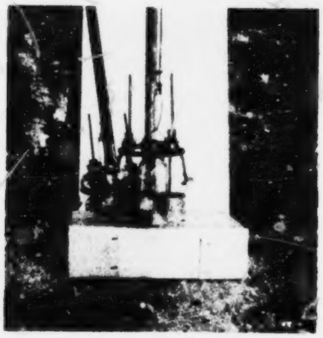
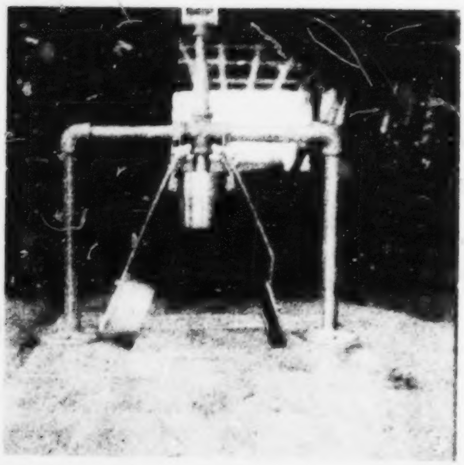


Figure 4 - Shallow water sampler



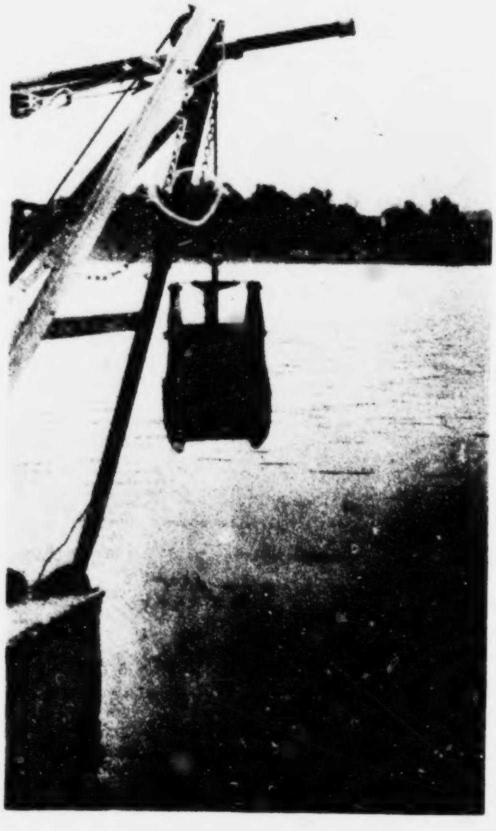


Figure 5 - Clam-shell mud dredge sampler

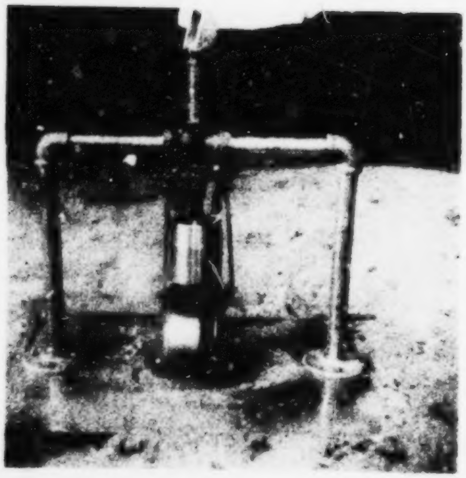


Figure 6 - Surface mud sampler, open and closed.

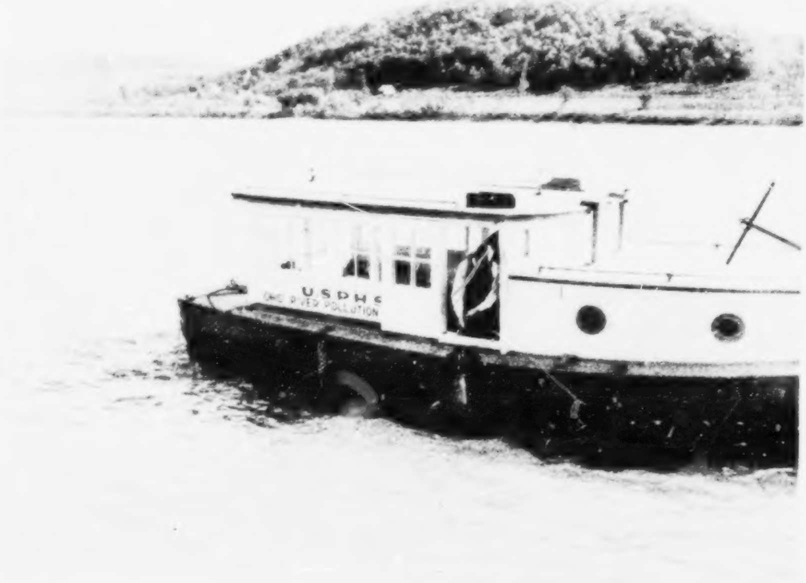
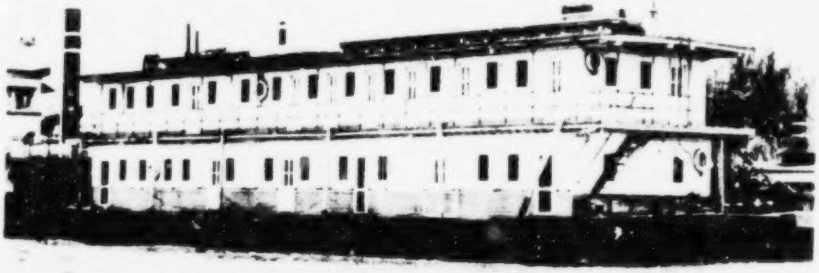

U.S.P.H.S.
OHIO RIVER POLLUTION

Figure 7 - Collecting a river water sample from a motorboat.



U.S. ARMY ENGINEER QUARTER BOAT "KISKI"
FLOATING LABORATORY
IN
OHIO RIVER POLLUTION STUDIES

Figure 8.

points, relocating them where local conditions indicated this to be desirable. The field engineer then prepared a detailed map showing the exact location of each sampling point and the highway routes necessary to reach it from the central location. This information, together with any other pertinent data, was turned over to the chemist in charge of each mobile unit, which then proceeded with its work in each location with a minimum loss of time. In Figure 9 is a typical guide map showing the location of a sampling point.

Each mobile laboratory was manned by a crew of three men, consisting of a chemist in charge, a laboratory attendant and a chauffeur-sample collector. In the accompanying map (Figure 10) are shown the travel routes and central location points followed by each mobile unit during the campaigns of 1939 and 1940. In Figures 11 to 13, inclusive, are typical exterior and interior views of a trailer laboratory. An interior floor plan is shown in Figure 14*.

In practice, the mobile laboratories usually were located at local water works or sewage treatment plants, where continuous supplies of water and electric current were available. These supplies were connected directly to the laboratory through extension hose and cable, forming part of the trailer equipment. Supplies of chemicals and apparatus were maintained by shipments from the headquarters laboratory at Cincinnati.

Laboratory Determinations and Methods

Every possible effort was made in the laboratory work of the Survey to obtain comparable results in accordance with current standard methods of water examinations. Recognizing that with several laboratories in operation, small variations in procedure might tend

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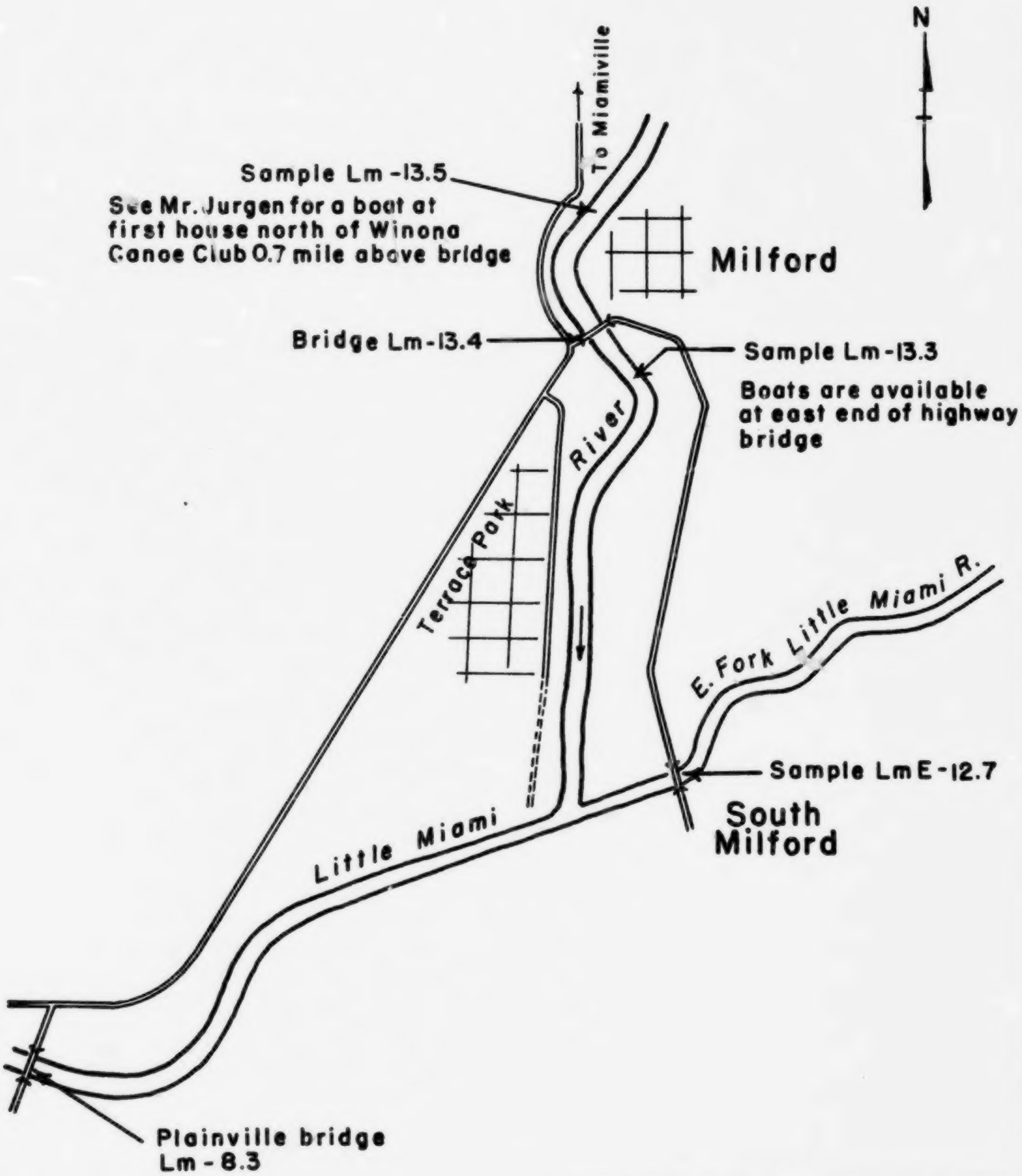
(*) For a detailed description of the trailer laboratories, see article on "Mobile Laboratory Units of the Ohio River Pollution Survey", by F. E. DeMartini. Public Health Reports, Vol. 56, No. 15, Apr. 11, 1941, pp 754-760 see Reprint No. 2259. Detailed specifications for these units are given in Appendix II of this supplement.

Figure-9

Typical Map of Sampling Point Locations


N
To Miamiville
Sample Lm-13.5
See Mr. Jurgen for a boat at first house north of Winona Canoe Club 0.7 mile above bridge
Milford
Bridge Lm-13.4
Sample Lm-13.3
Boats are available at east end of highway bridge
River
Terrace Park
E. Fork Little Miami R.
Sample LmE-12.7
South Milford
Little Miami
Plainville bridge Lm-8.3

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1942

Fig.-10

OHIO RIVER BASIN

LOCATION OF LABORATORY
UNITS DURING PROGRESS OF THE
OHIO RIVER POLLUTION SURVEY 1939-1941

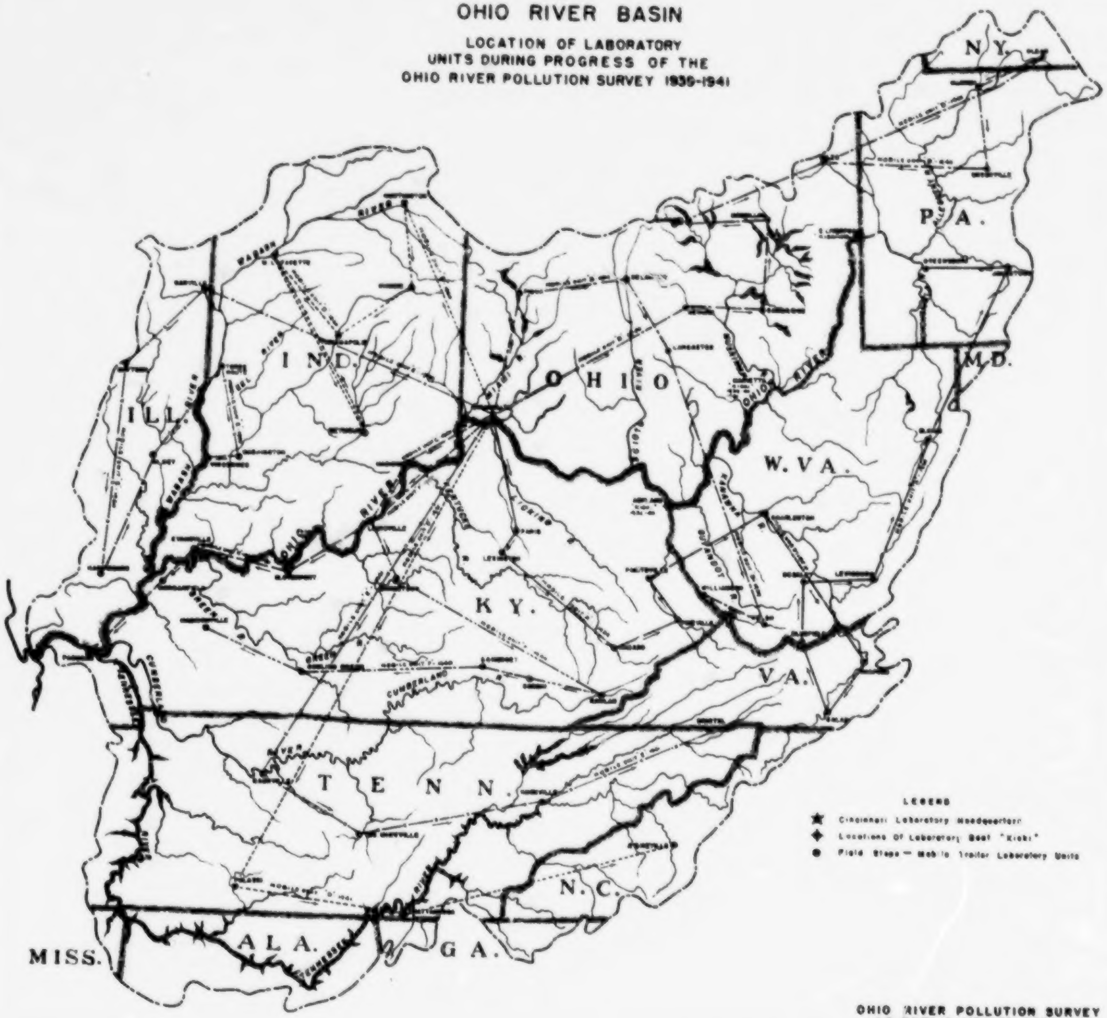
N.Y.
P.A.
M.D.
IND.
OHIO
ILL.
W. VA.
KY.
VA.
TENN.
N.C.
MISS.
ALA.
GA.
CUMBERLAND
LEGEND
★ Cincinnati Laboratory Headquarters
◆ Locations Of Laboratory Boat "Kiski"
● Field Stops — Mobile Trailer Laboratory Units

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Fig.-10

Figure 11 - Trailer laboratory, with towing car.

Figure 12 - Trailer laboratory in operation.

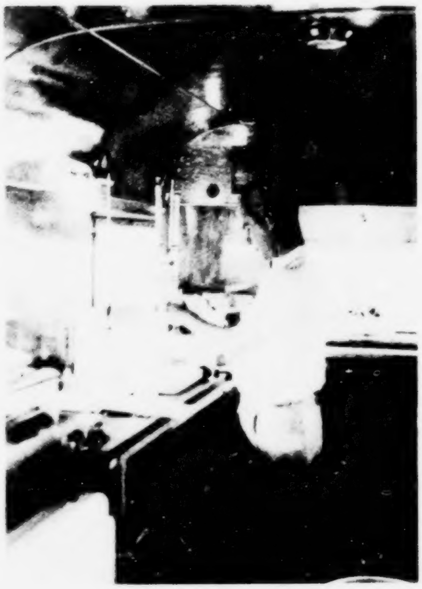
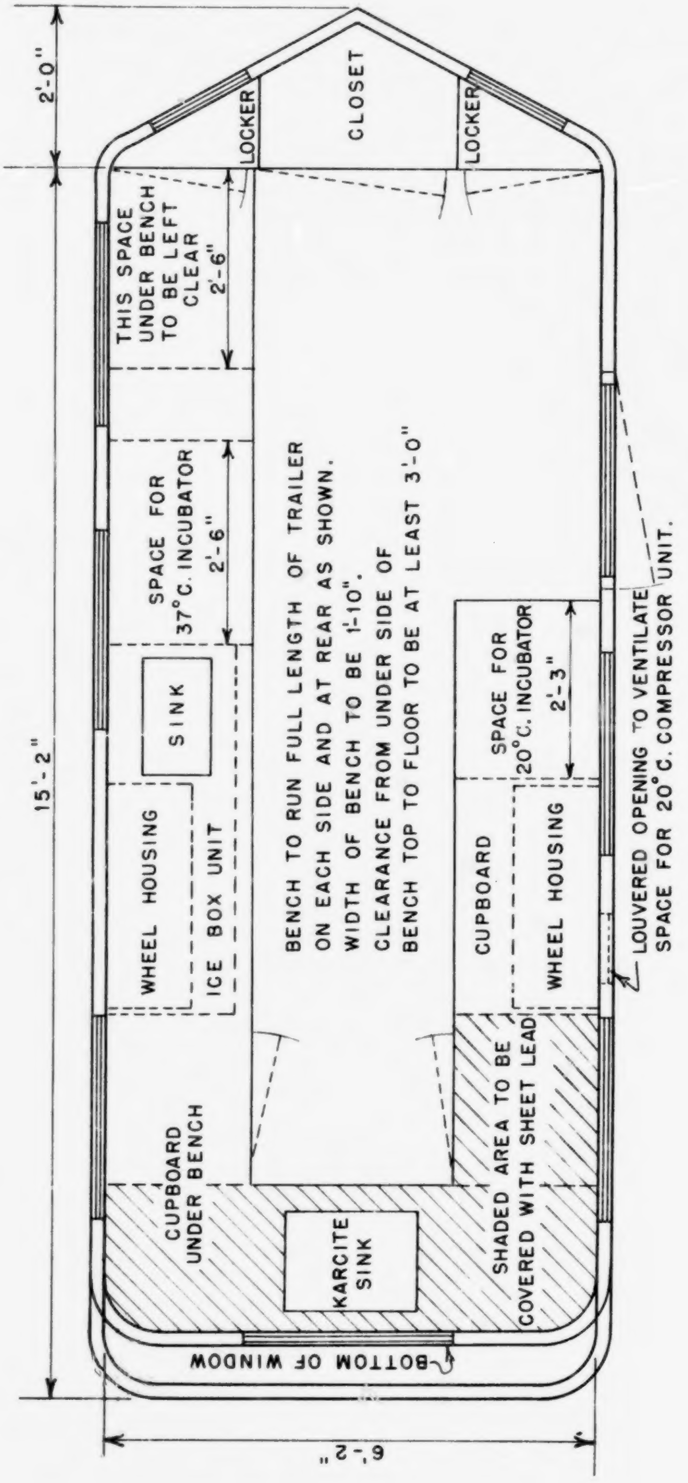


Figure 13 - Trailer laboratory, inside view.

Figure-14

Interior Plan of Trailer Laboratory


2'-0"
15'-2"
6'-2"
LOCKER
CLOSET
LOCKER
THIS SPACE UNDER BENCH TO BE LEFT CLEAR
2'-6"
SPACE FOR 37°C. INCUBATOR
2'-6"
SINK
WHEEL HOUSING
ICE BOX UNIT
BENCH TO RUN FULL LENGTH OF TRAILER ON EACH SIDE AND AT REAR AS SHOWN.
WIDTH OF BENCH TO BE 1'-10".
CLEARANCE FROM UNDER SIDE OF BENCH TOP TO FLOOR TO BE AT LEAST 3'-0"
SPACE FOR 20°C. INCUBATOR
2'-3"
CUPBOARD
WHEEL HOUSING
LOUVERED OPENING TO VENTILATE SPACE FOR 20°C. COMPRESSOR UNIT.
CUPBOARD UNDER BENCH
KARCITE SINK
SHADED AREA TO BE COVERED WITH SHEET LEAD
BOTTOM OF WINDOW

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1942

to vitiate the mutual comparability of the results, a systematic and thorough instruction in laboratory methods was given to each technician at the Cincinnati headquarters laboratory before his assignment to regular duty. This course of instruction usually occupied from four to six weeks. Throughout the progress of the field work, a qualified member of the laboratory staff was detailed to visit in turn each base and mobile laboratory in order to check analytical methods and results and to correct any inconsistencies.

In this connection, detailed instructions also were given to the chemists in charge of mobile laboratory units concerning the management of these units. In this connection reference is made to Appendix I, Memorandum No. 11, which contains information prepared especially for the mobile laboratory crews. In Memorandum No. 2 of the same Appendix is a general statement of the plan and objectives of the survey which was prepared for the guidance of all of the members of the laboratory staff.

The routine laboratory tests made on stream water samples were as follows:

A. Physical and chemical tests: (See Appendix I, Memorandum No. 4)

(1) Temperature; (2) turbidity; (3) pH value; (4) alkalinity; (5)* total and volatile suspended matter; (6) dissolved oxygen; and (7) 5-Day biochemical oxygen demand at 20° C.

B. Bacteriological tests: (See Appendix I, Memorandum No. 3)

(1)* 24-hour agar plate count at 37° C.; (2) coliform bacteria, "most probable numbers", by standard fermentation tube test at 37° C.; (3)* direct plate count of coliform bacteria on brilliant green lactose bile medium at 37° C.

(*) Discontinued as a routine test after the end of the year 1939.

The following brief notes may be inserted at this point to explain the significance of the various tests above enumerated.

A. Physical and Chemical Tests.

(1) Temperature

The temperature of stream waters governs their solubility for oxygen and hence the saturation level of dissolved oxygen in streams. This saturation level varies inversely with the stream temperature, being lower at higher temperatures and vice versa. Temperature also has a marked influence on rates of natural purification, which are increased at higher temperatures and diminished at lower temperatures.

(2) Turbidity

In stream waters, turbidity is measured in terms of parts per million (p.p.m.) or milligrams per liter, of a standard suspension of diatomaceous earth. It is an index of the density of silt, or other suspended matter, carried by a stream.

(3) pH value

The symbol "pH" denotes the logarithm of the reciprocal of the hydrogen ion concentration in a given water, this symbol being a universal one in chemistry. In general, the pH value indicates the relative acidity or alkalinity of a water, being lower with higher degrees of acidity. The normal value for neutral distilled water is 7.2, higher values indicating the presence of alkaline earth salts and lower values, the presence of acids or acid salts.

(4) Alkalinity

The alkalinity of a natural water represents its content of carbonates, bicarbonates, hydroxides, and occasionally borates, silicates and phosphates. It is measured by titration with a standard solution of a strong acid to certain standard datum points or hydrogen ion concentrations. In the present survey, standard sulphuric acid has been used, with methyl orange as an indicator, showing a definite color change at a hydrogen ion concentration of 0.0001 (or pH 4.0).

(5) Total and volatile suspended matter

The total suspended matter content of a natural water is determined by filtering a sample of standard volume and weighing the dried residue. It represents the concentration of suspended matter in terms of dry solids. The volatile matter is determined by the loss of weight in the total dry solids after the application of heat under standard time and temperature conditions. It is a measure of the suspended organic matters present in the water.

(6) Dissolved oxygen

Although oxygen is only slightly soluble in water (about 9.2 p.p.m. at 20° C.), it can be measured accurately to less than 0.1 p.p.m. Dissolved oxygen is essential to the natural purification of stream waters and to the maintenance of fish and other aquatic life. In natural bodies of water, the dissolved oxygen is drawn upon to support biochemical oxidation of organic waste matters, but tends to be replenished by absorption from the atmosphere and the photosynthetic action of some water plants, including algae. A deficiency in the dissolved oxygen content of a polluted stream below the saturation level indicates the presence of polluting organic substances which are absorbing oxygen from the stream water. The degree of this deficiency is a measure of the deoxygenating effect of polluting matters and hence an index of the degree of pollution in a particular stream zone.

In an ideal situation in which a stream receives sewage or industrial waste at a single point and rapid admixture with the stream water takes place, the dissolved oxygen content tends to follow a typical "sag" curve on the basis of time and temperature, reaching a minimum point usually in one to three days time of flow below the source of pollution, depending on the temperature, oxygen demand and rate of reaeration. The specific rate of atmospheric reaeration is influenced slightly by stream temperature but largely by the turbulence of flow, which varies widely in different streams, or sections of the same stream. The minimum point of the "oxygen sag" curve is perhaps its most important parameter from an observational standpoint, as it marks the most unfavorable condition which may affect fish and other aquatic life, or the possibility of occurrence of "nuisance" conditions in a stream. Desirable limiting requirements in this oxygen minimum point are discussed in the main report of the survey.*

(7) Five-day biochemical oxygen demand (B.O.D.)

The standard test for biochemical oxygen demand (B.O.D.) involves the incubation of sealed samples of a stream water for 5 days at 20° C. and the measurement of the loss of dissolved oxygen by the sample during the period of incubation. This loss represents the 5-day biochemical oxygen demand of the sample. When diluted initially with B.O.D.-free water, the oxygen demand of the original sample is found by applying the dilution ratio to the measured loss of dissolved oxygen.

The B.O.D. as thus determined is a measure of the amount of dissolved oxygen which may be expected to be absorbed from a stream water in 5 days at 20° C. in order to support the biochemical oxidation of the organic pollutants carried in the stream at the time of observation. Organic matters originating in sewage have been found experimentally to be oxidized in this manner according to a logarithmic time-function curve, which varies in its rate of progression with the water temperature, the rate being faster at higher temperatures. Hence it is possible to estimate the amount of oxygen demand satisfied in any time and at any temperature, having observed it at a standard time and temperature.

B. Bacteriological Tests.

(1) 24-hour plate count at 37° C.

This determination consists in mixing a measured portion of the water sample with a melted sterile culture medium containing agar, spreading the mixture in a sterile glass petri dish, allowing it to become hardened to a

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(*) See pages 32-35, Introductory and General Sections, Report to Ohio River Committee.

stiff, jelly-like consistency and incubating the hardened culture for 24 hours at 37° C. At the end of this period, visible colonies formed from individual or clumped bacterial cells are counted and their number is taken as an index of the density of bacteria in the original sample. As the species of water bacteria appearing on these plate cultures are numerous and varied, the bacterial count obtained in this manner is only roughly indicative of pollution. When considered in conjunction with the determination of numbers of coliform bacteria, the plate count is of value, both as an indicator of pollution and as a rough measure of natural purification.

(2) Determination of coliform bacteria

This determination affords the most delicate and specific test for pollution of stream waters by sewage, as it shows the approximate density of a group of bacteria which are always present in large numbers in sewage and are relatively few in numbers in other stream pollutants. Coliform bacteria are normal inhabitants of the intestines of warm-blooded animals and are discharged in very large numbers in human feces, which constitute the principal source of these bacteria in sewage.

The test for coliform bacteria is made by adding measured portions of a water sample to a lactose broth liquid culture medium in tubes especially designed for showing gaseous fermentation of the lactose. After 24 to 48 hours of incubation at 37° C., tubes showing the presence of gas are considered as giving presumptive evidence of the presence of coliform bacteria, which are lactose fermenters. After the necessary confirmatory tests, the result is recorded as positive or negative. The "most probable number" (M.P.N.) of coliform bacteria is determined from the numbers of tubes giving a positive result with different volumes or dilutions of the original sample. The principle underlying this method of enumeration is based on the theory of probability, a given result having a definite maximum probability that a definite number of coliform bacteria per unit of volume (i.e. a milliliter or 100 milliliters) is present in the sample tested. Although this method is subject to a considerable error in single determinations, it probably affords the most logical and relatively precise one available for determining small densities of coliform bacteria from fermentation tests.

(3) Direct plate enumeration of coliform bacteria

This method, originally developed in its present form by Noble and Tonney, utilizes the plate-count procedure described above under B-(1), but depends on the use of a culture medium which is selective for bacteria of the coliform group. Experience with its application to a large number of water examinations in the present survey has indicated that it gives average results which agree fairly well with those of the fermentation test, though individual results may show considerable divergence. The relative precision of the direct count seems to vary to some extent with the density of coliform bacteria present in the sample and also with the number of countable colonies on the plates. A certain degree of skill, resulting from experience, is necessary for the identification of colonies on the plates which are typical of coliform bacteria. The culture medium is only partly selective as it permits the growth of a limited range of non-coliform species.

Additional tests of a special nature, made only on certain samples, have included nitrite, nitrate, acidity to phenolphthalein (hot and cold), and total hardness. In some cases routine tests for turbidity and alkalinity have been limited to samples from certain key points. Biological tests have been carried out as indicated in the section of this report devoted to the biological survey work. (Appendix 1, Memorandum No. 8)

From time to time it has been desirable to examine certain types of industrial wastes concerning which little has been known of their composition and more especially as to their suspended and volatile matter content and biochemical oxygen demand. These tests have been made as required, both at the base and at the mobile laboratories.

During the year 1940 considerable numbers of samples of bottom mud deposits collected from the Ohio River were examined at the Cincinnati base laboratory. The tests made on these samples included biochemical oxygen demand after 2, 4, 6, 8 and 10 days, total and volatile matter, moisture content, and amounts of potassium permanganate and bichromate consumed under standard time and temperature conditions.

In connection with the mobile laboratory operations, a regular practice was made of examining bacteriologically the more important public water supplies of communities visited by these laboratories. At the Kiski laboratory and in connection with the special study of taste and odor difficulties in water supplies along the Mahoning and Beaver Rivers, numerous routine tests for phenol content were made on stream waters suspected of being involved in these difficulties.

Summary of Routine Laboratory Operations

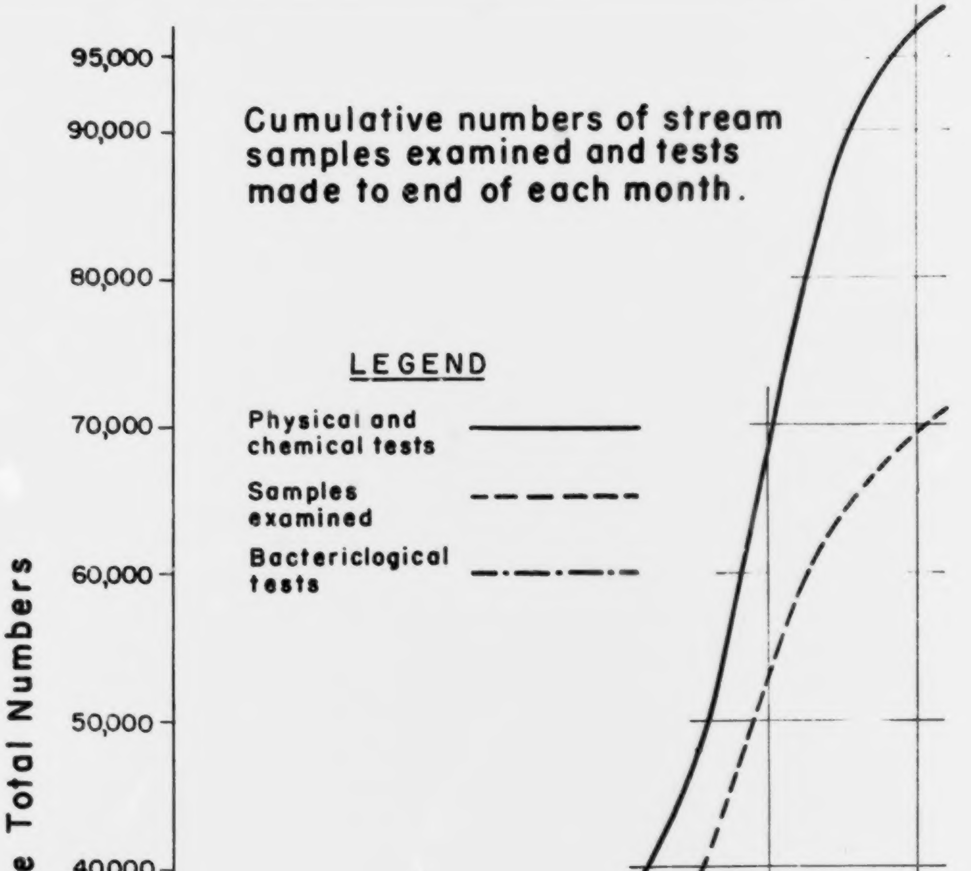
Up to the end of March, 1941, when routine laboratory operations were terminated, a total number of 71,124 water samples had been collected and examined in connection with the Survey, exclusive of samples examined biologically. The total number of examinations made on these samples was 131,132, of which 98,554 were physical and chemical tests and 32,578 were bacteriological examinations. The average number of samples examined during the 26 months of the laboratory operations was about 2640 per month, with a maximum number of 6570 in August, 1940, when operations were at their highest intensity. The number of laboratory tests averaged about 5,000 per month for the entire period of the Survey and reached a maximum number of 11,420 in August, 1940.

In Figure 15 are shown graphically the total number of samples examined and tests made up to the end of each successive month, beginning with January, 1939, when a few samples were collected from the Ohio River at Cincinnati. The effect of winter curtailment of the stream examinations, owing to adverse weather conditions, is apparent in the trend of the curves during the December-February period of 1939-40. Part of the lag in the upward trend of the curves shown for this period was due, however, to the reorganization incidental to carrying out the accelerated plan of operations adopted in February, 1940. The lag near the end of the survey period indicates the effect of curtailing operations gradually, beginning in October, 1940.

On the basis of records maintained throughout the progress of the survey, the average amount of work performed by one mobile laboratory unit per month was as follows:*

(*) Figures taken from article by F. E. DeMartini, previously cited.

Fig.-15



Cumulative numbers of stream samples examined and tests made to end of each month.

OHIO RIVER POLLUTION SURVEY
U.S. PUBLIC HEALTH SERVICE
1942

	Mobile Laboratories	Kiski Laboratory	Cincinnati Laboratory
Cost per sample	$2.78	$2.26	$1.79
" " test	1.22	1.13	1.09

The somewhat higher unit cost of operating the mobile laboratories was due mainly to the conditions of their operation, which necessitated moving them from place to place about every two weeks, thus entailing some degree of interruption in the work, whereas the base laboratories were operated on a continuous schedule, with no interruptions due to extraneous circumstances. Another important element in the higher cost of operating the mobile laboratories was the added expense of maintaining their personnel in the field, which included an extra per diem allowance for subsistence. The base laboratory personnel were located at fixed stations and did not receive such an allowance. In the absence of this added item of expense, the mobile laboratories would have shown a considerably lower unit cost of operation, because less capital and upkeep expense was involved in providing and maintaining them. This type of laboratory was found to possess many practical advantages over those of fixed location in covering a large and varied stream area. It was in some respects the most valuable new device for facilitating stream pollution surveys which was developed in the present survey.

Personnel

The laboratory operations of the Survey were performed under the administrative direction of Senior Sanitary Engineer J. K. Hoskins, in charge of the Stream Pollution Investigations Station at Cincinnati, who was succeeded in July, 1940, by Medical Director H. E. Hasseltine. The work was organized and carried out under the technical direction of Senior Sanitary Engineer H. W. Streeter, with Biologist F. J. Brinley in immediate charge of biological studies, Public Health Engineer C. T. Carnahan and Passed Assistant Sanitary Engineer F. E. DeMartini in charge of mobile field laboratories, and Public Health Engineer S. G. Monroe in charge of the floating base laboratory "Kiski". Principal Bacteriologist C. T. Butterfield, Principal Chemist C. C. Ruchhoft, Special Expert W. C. Purdy and

Senior Biologist J. B. Lackey, all from the regular staff of the Cincinnati station, cooperated actively in training the laboratory personnel, preparing instruction memoranda on laboratory methods and in acting as technical advisors in various phases of the work. Passed Assistant Sanitary Engineer (R) C. L. Chapman was in charge of the special acid stream work based at Morgantown, W. Va. The six mobile laboratories were under the immediate charge of Junior Chemists Stuart Cohen, Stephen Megregian, F. W. Middleton, J. I. Norris, O. G. Pettijohn and R. A. Snider. The laboratory work at the Kiski laboratory was performed by Assistant Chemist W. W. Walker and Bacteriologist B. S. Levine, and at the Cincinnati laboratory, by Assistant Chemist M. B. Ettinger and Junior Bacteriologist C. W. Chambers.

Acknowledgements

The laboratory work of the Survey was carried out with the active collaboration and assistance of several agencies which were most helpful in reinforcing the work of the regular staff. In the biological work, active collaboration was furnished by the laboratory of Interior Fisheries Investigations, U. S. Bureau of Fisheries, under the direction of Dr. M. M. Ellis, in examining numerous fish specimens as to their physiological condition and preparing a report of this work which is included as appendix to the general report of the biological investigations (See Supplement F, to the main report of the Survey). Cooperation in the biological work on fish life also was given by the Ohio Division of Conservation through Messrs. E. L. Wickliff and M. B. Trautman of that staff. The laboratory of the Dayton, Ohio sewage treatment plant, under the direction of Mr. M. W. Tatlock, carried out systematic examinations of stream waters in that vicinity as a direct contribution to the work of the Survey. The laboratories of the Tennessee Valley Authority and the Tennessee Department of Health made available the results of their extensive stream analyses in the Tennessee and Cumberland river basins, respectively. To all of these agencies and individuals grateful acknowledgement is due for their generous cooperation, which greatly facilitated the work of the survey and made possible certain economies in the scope of operations. Acknowledgement also is due to the various municipal officials, too numerous to mention

here, who assisted in providing location places for the trailer laboratories during their field operations, and to the State Sanitary Engineering Divisions which facilitated these arrangements. Finally, special acknowledgement should be made of the courteous assistance furnished by the offices of the Ohio River Division and of the District Engineers, Corps of Engineers of the U. S. Army, in arranging for the collection of river water samples at the navigation dams in the Ohio River and for the provision of various services and material supplies in connection with the operations.

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APPENDIX I

LABORATORY MEMORANDA

LABORATORY MEMORANDA

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Memorandum No. 1

December 15, 1938.

Collection of River Water Samples at U. S. Locks & Dams

Memorandum No. 1

December 15, 1938.

COLLECTION OF RIVER WATER SAMPLES AT U. S. LOCKS & DAMS

The following instructions have been prepared for the use of U.S. Engineer Corps personnel who may be assigned to the regular collection of river water samples at the Ohio River locks and dams and at the mouths of certain tributaries.

Sampling Equipment

The regular sampling equipment (sampler will consist of a bronze subsurface collecting vessel, fitted with a rope for lowering and raising the vessel and a heavy cord for operating the release cock. The hoisting rope will be marked at 5-foot intervals with colored twine. It is assumed that the collector will be provided with a skiff and out-board motor for use in collecting the samples.

The sampler is fitted with a cover, gasket and thumb-screw lugs for tightening the cover in place. A raised platform inside the sampler is provided with clips for holding three bottles. Two of these bottles are identical in shape and size, being intended for collecting duplicate samples for dissolved oxygen and biochemical oxygen demand tests. The third bottle (sterilized) is for the bacterial sample. In the cover of the sampler are tubes located so that they may be extended down into the three bottles when the cover is in place. One of these is a sterilized glass tube for the bacterial sample bottle. This tube will be changed for each collection. When the cover is locked in place, the tubes will project down into the bottles through their open mouths, the stoppers being removed when placing the bottles into the clips. A supply of bottles, with carrying cases, and glass tubes will be furnished the collector in advance.

Location of Sample Collection Points

Pending the more exact location of sampling points in the river and establishment of ranges for locating these points, samples will be collected at three points on a cross-sectional line extending across the channel about 500 feet upstream from each dam and in a direction parallel to the dam. One of the three points will be located approximately mid-way across the stream and the other two at points located approximately mid-way between this center point and the shore in each direction. An effort will be made to obtain each sample at about mid-depth in the stream; that is, at a point about mid-way between the surface of the water and the bottom of the channel.

As soon as possible after the regular collection of samples has been started, U. S. Public Health Service engineers will locate permanent sampling cross-sections above and below each dam and will establish on each cross-section convenient range lines whereby the collector will be able to locate himself readily on each point. At the same time soundings will be made to establish the river depth at each point corresponding to pool stage or to some other known stage of the river and the corrected depth at other stages can then be determined by adding or subtracting 1/2 the number of feet which a given river stage is above or below the reference stage (i.e. pool or otherwise). Temporarily, the depth at each point can be established roughly by sounding with the collecting vessel, or with a sounding line, and then bringing the vessel to about one-half this depth.

Method of Collecting Samples

Before starting the collection each set of three sample bottles will be marked as "R", "C", or "L", denoting respectively the right, center and left points along the cross-section, facing downstream. In reaching the first point, whether it be the right (R) or left (L), the three bottles thus designated will be placed in the clips in the sampler with their stoppers removed (and remaining tied to the necks of the two identical bottles). The bacterial bottle will be wrapped in paper as delivered to the collector and will be sterilized before delivery, as it is intended for collecting a sample for bacteriological examination. The stopper of this bottle will be covered with tin-foil, which should not be removed in taking out the stopper. In handling this bottle and its stopper, the greatest care should be taken by the collector not to touch with hisfingers or anything else the inside of the bottle neck or the corresponding outer face of the groundglass stopper, or to lay the stopper down so that this face comes in contact with any surface. The object of this precaution is to prevent any contamination of the sample bottle from an outside source during collection of the sample.

The stopper should be handled with the tin-foil cover in place, so as to facilitate protecting it from contamination.

In inserting the sterilized glass tube for this bottle into the cover of the sampler, similar precaution should be taken not to allow the fingers to come into contact with the tube. By removing only a part of the wrapper from the tube, the bare end can be inserted in the cover by holding the covered end in the fingers.

After the bottles have been placed in the clips and the glass tube inserted in the cover, the cover is then placed on the sampler with the tubes projecting into the bottles, the thumb-screw lugs tightened into place and the air-release cock closed, with the handle turned to a horizontal position. The sampler is then lowered to mid-depth and the air-release cock opened by pulling the auxiliary cord. Water then will flow into the sampler until the air vent is trapped by the short tube. When the flow is stopped, the two duplicate bottles should be completely full and the third (bacterial) bottle, 1/2 to 3/4 full. During the filling operation, displaced air from inside the sampler will escape and appear at the surface of the river as bubbles. As soon as this air bubbling ceases, the vessel is ready to draw up to the surface and be lifted into the boat (it should be kept in a vertical position so far as possible while lifting it into the boat).

After removing the cover, the single bacterial sample bottle is removed first and its contents brought down, if necessary, to a level of about 3/4 full. The stopper is inserted with the same precautions against contamination by the fingers as above noted. This sample is then wrapped in its paper, with the tin-foil cover in place, and placed vertically in the carrying case. The two duplicate bottles are then removed, completely full, and their stoppers inserted so as not to allow any air to be trapped under them in the neck of the bottle. If water should be spilled from these bottles while removing them from the sampling vessel, they should be filled to the top with water drawn from the sampler with a glass pipette, before inserting the stoppers. These bottles, with their stoppers tightly inserted, will then be placed in the carrying case and the collector will proceed to the next sampling point, where the same operations as above described will be repeated.

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Transporation of Samples to Laboratory

As a rule, the samples after collection will be taken by the collector to a designated point near the dam and there picked up by the U. S. Public Health Service collector, who will transport them in his automobile to the laboratory. As the automobile transportation route will follow the right (Ohio-Indiana) bank of the river, arrangements will be necessary for delivering the samples to convenient points along that side. As soon as the automobile transportation has become established the collection of the samples from the river can be timed so as to involve a minimum of delay between the time of collection and the time of being taken up for transportation to the laboratory. Ordinarily, the working schedule will provide for the collection of a set of samples once each working day, or every other day, at some time between 3:30 A.M. and 9:00 A.M., depending on the distance of a particular dam from the laboratory and its position on the automobile transporation route. After this time has been established, it will be subject to little if any variation from day to day. During the warmer season, the samples after collection will be placed and kept in iced containers (to be provided by the laboratory) from the time of collection to the time of reaching the laboratory. This precaution is necessary in order to prevent multiplication of bacteria in the samples, with corresponding changes in their numbers, during transportation to the laboratory.

When stopping at each dam for the samples, the U. S. Public Health Service collector will leave a complete set of sample bottles with containers for the next collection. As a rule, an extra set of bottles will be kept at the dam for use when for any reason a set may not be delivered in time for the next collection. Each sample collector will be provided with a complete set of river sampling equipment which he will keep in a convenient place at the dam.

Special Samples

Occasionally, in order to meet the needs of the laboratory work, it will be necessary to request an extra sample of river water for transportation to the laboratory. Samples of this nature usually can be supplied from the surplus water in the sampler, using extra glass containers which will be furnished by the laboratory.

At certain dams, it will be necessary occasionally to request the collection of two sets of samples at one time, one being collected above the dam, as usual, and the other at least one-half mile below the dam (so as to remove the effect of any entrained air). Where the mouths of important tributaries are located near dams, regular collection of a single mid-point sample from these tributary mouths may be requested, in addition to the set of samples from the main stream.

At some dams, notably Nos. 27, 29, 31, 37 and 38, a study will be undertaken of the nature of sludge deposits preceding and during periods of low water, when the effects of sedimentation will be most apparent. In this connection, it may be necessary to request the occasional collection of mud samples from the river bottom, using special equipment which will be provided by the laboratory. This will not entail any material increase in the time required for the collector's services, as collection of these samples can be made at the same time as that of the regular river water samples. It will involve no difficulties other than the occasional handling of the mud collection equipment.

APPENDIX - MEMORANDUM NO. 1

DETAILED INSTRUCTIONS TO SAMPLE COLLECTORS.

1. For collecting one complete set of samples of river water on a cross-section of the river, the following equipment will be provided to each collector:

(a) One deep-water sampler with lid, gasket, inside platform with clips, hoisting rope marked at 5-foot intervals and air-release valve with separate operating cord.

(b) Nine (9) bottles, of which six (6) are narrow-mouth, unwrapped, and three (3) are wide-mouth, wrapped (sterilized).

(c) An extra supply of bottles in case of breakage; also any extra bottles for special samples as required.

(d) Sterilized glass tubes in wrappers.

(e) A water temperature thermometer, mounted in a nonbreakage rod.

(f) A glass pipette and funnel.

(g) Carrying case for sample bottles and samples.

2. For the ordinary collection, a set of three (3) bottles will be collected at each one of the three (3) points on a river cross-section. For convenience, these three points will be designated by the letters (R), (C) and (L), denoting respectively the right, center and left points on the section, facing downstream. Each set of three bottles will consist of two narrow-mouth bottles (duplicates) and one wide-mouth bottle. Each set should be marked with its proper letter corresponding to the point of collection.

3. Locations of (R), (C) and (L) points will be furnished each collector in advance, mainly by means of shore ranges whereby he may locate himself on a given point in a boat under any river condition. Pending more exact location of these ranges, samples will be collected on a cross-section located

about 500 to 1000 feet above each dam, the (C) point being about halfway across the channel and the (R) and (L) points midway between the (C) point and the shore in either direction. Samples at each point will be collected at approximately mid-depth. Temporary ranges for these cross-sections will be established for the collectors, using two prominent landmarks, one on each side of the river, lined approximately at a right angle to the direction of the flow.

4. Before proceeding to the first collection point, the collector should place a set of three bottles in the sampler clips and insert a fresh sterilized glass tube into the hole provided on the under side of the sampler cover. Before placing the bottles the stoppers should be removed, those in the duplicate narrow-mouth bottles being left tied to the bottle neck and the stopper of the wide-mouth bottle being carefully removed with the tinfoil in place and inverted resting on its top at some convenient place in the boat.

Caution: In handling the wrapped wide-mouth bottle, do not allow the fingers to touch the inside of the bottle or its neck, or the ground face of the stopper. Save the wrapper for wrapping the sample after collection. In inserting the glass tube, do not allow the fingers to touch the tube itself. Handle one end with the wrapper in place,inserting the bare end.

5. With the bottles in place, the cover is then set on the sampler, with the two metal tubes projecting down into the narrow-mouth bottles and the glass tube into the wide-mouth bottle. Tighten the cover securely by means of the six thumbscrew lugs, with the gasket in place, so as to make a tight joint. The air-release valve should be closed, with its handle in a horizontal position.

6. The sampler is next lowered over the side of the boat into the river, being careful not to allow the air valve cord to become fouled with the hoisting rope. Using the 5-foot markers, the sampler is lowered to about mid-depth and while held in this position the air-valve cord is pulled smartly, so as to open the valve. As soon as this valve is open, water will flow into the sampler through the tubes and fill the bottles by several displacements, the air escaping through the air-valve and appearing as bubbles at the surface. As soon as these bubbles have stopped coming up to the surface, the sampler may then be hoisted back into the boat.

7. With the filled sampler in the boat remove the cover and place the thermometer in the water in the vessel, leaving it there for a later reading. Next, take out the wide-mouth

bottle, which should be about three-quarters full, insert the stopper carefully and restore the wrapper, being sure that it is properly marked with the letter designating the point and the number representing the location of the cross-section (this number will be furnished to the collector). Then remove the two narrow-mouth bottles, which should be completely full, and insert their stoppers so that no bubbles of air will be left in the bottle under the stopper. If the bottle is not completely full, draw a little water from the sampler vessel with the pipette and fill it in this way. Then read and record the temperature of the water as shown by the thermometer.

8. From the surplus river water left in the collector, remove enough to make up any special sample required and pour the rest overboard. A glass funnel will be furnished for use in pouring water from the sampler into an extra bottle if required.

9. Now proceed to the next two points and repeat operations (4), (5), (6), (7) and (8) at each point.

10. After each collection, place the samples in the carrying case, being sure that they are marked properly. Place the discarded glass tubes in the case and secure the cover of the case, ready for transportation to the designated point on the shore.

11. After finishing the day's collection the sampler should be flushed out with clean water, the metal tubes and cover wiped clean and the rope and cord coiled neatly, ready for the next collection. The sampler and other equipment should be stored at a suitable place near the boat mooring. Directions will be given each collector as to where the carrying case containing the samples should be delivered after collection.

12. During the warm season, provision may be necessary for packing the samples in an iced container for transportation to the laboratory. These arrangements will depend on circumstances and on the availability of a small amount of ice locally. The iced containers will be furnished from the laboratory when their use is necessary.

13. In collecting samples from tributaries, where required, a single point in the middle of the stream will be used, rather than three points as in the main river. Samples will be collected at mid-depth in tributaries as in the main stream.

Memorandum No. 2

December 29, 1938

Plan And Objectives of Laboratory Survey

39-A

Memorandum No. 2

December 29, 1938.

PLAN AND OBJECTIVES OF LABORATORY SURVEY

This memorandum has been prepared especially for the information of new personnel who have not been connected with previous work of the U. S. Public Health Service and have not been acquainted with the history, plan and objectives of the present survey of pollution of the Ohio River and its tributaries. It is hoped that through this medium those who will be actively engaged in the work, and especially in its laboratory side, will be enabled to gain a better perspective of the work as a whole and of the respective parts which they will play in it. It is only through such an understanding that individual effort may be intelligently directed to the particular work in hand and that a sense of pride and "esprit de corps" may be developed among those who will be collaborating in the very considerable task that lies ahead.

Historical Background of Present Survey

In the year 1912, an Act of Congress extended the functions of the Public Health Service and authorized investigations of "the diseases of man and conditions influencing the propagation and spread thereof, including sanitation and sewage and the pollution, either directly or indirectly of the navigable streams and lakes of the United States." In 1913 a special appropriation, continued annually since that year, was made for carrying out these provisions, including investigations of stream pollution. In the summer of 1913 work was begun on a study of the pollution and natural purification of the Ohio River, which was selected as a typical large inland stream receiving sewage, mostly untreated, and also used very largely as a source of public water supplies. This study was continued until the end of 1916.

At the time of this study, little was definitely known concerning certain fundamental quantitative relationships which are involved in the pollution and self-purification of rivers. The main objective of the study was to obtain some basic observations bearing on those relationships, such as the ratios of measurable sewage pollution to numbers of contributing population, the polluting effects of certain industrial wastes in terms of quantities of manufactured products and of equivalent sewage-contributing populations, and the various effects of time, temperature, pollution density, etc. on observable rates of natural purification of sewage-polluted streams. Incidentally, a fairly comprehensive general picture was obtained of the status of pollution of the Ohio River proper in zones extending below certain major centers of pollution, such as Pittsburgh, Wheeling, Cincinnati and Louisville, and also of the more important tributaries at their points of entry into the Ohio. The observations did not extend to all sections of the Ohio River or to any of its tributary streams except at their mouths.

Interrupted by the World War, stream pollution activities of the Public Health Service were resumed in 1921 with a study of the Illinois River, followed by surveys in the upper Mississippi, the lower end of Lake Michigan and, in 1930-31, a resurvey of that portion of the Ohio extending from above Cincinnati to below Louisville. Although these several investigations indicated conditions of pollution in the waterways concerned, they were primarily research projects designed to throw further light on the more basic problems of river sanitation. These field studies have been supplemented by basic research work at the Cincinnati laboratory dealing with certain problems of water and sewage purification closely allied with those of stream pollution. In this category have been studies of the efficiency and limitations of water purification and of the mechanism of sewage oxidation by activated sludge.

The Present Survey

The present survey has resulted from the River and Harbor Act of August, 1937, Section 5 of which provides that the Secretary of War is authorized and directed "to cause a survey to be made of the Ohio River and its tributaries to ascertain what pollutive substances are being deposited, directly or indirectly, therein and the sources and extent of such deposits and with a view to determining the most feasible method of correcting and eliminating the pollution of these streams." It is also provided in the Act that the survey shall include comprehensive investigations of the various problems relating to stream pollution and its prevention and abatement; that cooperation and assistance of the Public Health Service may be secured, that a report shall be made to Congress with recommendations for remedial legislation, and that the expenses of the surveys shall be paid from current appropriations for rivers and harbors work.

Shortly after passage of this Act, a request for active collaboration in the survey was received and accepted by the Public Health Service. As the result of preliminary conferences it was agreed that the Public Health Service would carry out the following parts of the study: (1) necessary laboratory studies, chemical and biological; (2) collection and study of factual data relative to pollution; (3) determination of the character of water in the main stream and its tributaries; (4) determination of the extent of treatment of polluting material required and (5) all other investigations necessary to determine existing conditions and, as far as possible, future conditions which may have bearing on the pollution problem. A lump-sum allotment of funds has been made to the Public Health Service by the War Department for carrying out this general program of work, a portion being made available for the current fiscal year.

Projected Work for the Current Year

For the current year, the proposed work will consist of:

1. Laboratory studies of the Ohio River proper between the mouth of the Kanawha and the mouth of the Kentucky River to determine existing conditions of pollution.

2. A study of the extent and effect of organic sludge deposits in the existing pools and especially in those formed by permanent dams.

3. Laboratory study of streams in the coal mining area to determine the amount of acid and acid salts carried by the river.

4. Surveys of the tributary streams entering the Ohio River between the Kanawha and Kentucky Rivers to determine the extent of pollution and remedial measures necessary.

5. Continuing surveys of the pollution of the Tennessee and Cumberland Rivers now under way, in cooperation with the Tennessee Valley Authority and the State Department of Health of Tennessee.

SURVEYS OF SOURCES OF POLLUTION

The work of the Survey has divided itself quite naturally into two main sections, one consisting of field surveys of sources of pollution and the other, laboratory surveys of the streams. The first section of the work, which is being organized as a separate project under Mr. Crohurst and Mr. Tisdale, will involve the systematic collection of data from state departments of health and other agencies bearing on sewered populations along the various streams, the amounts and kinds of wastes discharged by industries, the extent of sewage and industrial wastes treatment and allied matters. This survey also will disclose the locations of stream zones in which conditions of pollution are bad or unsatisfactory, thus pointing toward areas which may require some degree of laboratory study. It also will be concerned with the collection of basic data for later engineering studies of the interception and treatment of sewage from various areas of pollution.

HYDROMETRIC DATA

An essential part of the basic data for a survey of river pollution consists of detailed information bearing on the volumes of flow of a main stream and its tributaries, not only throughout the period of the survey, but also during a term of previous years, in order to establish the relative normality of various flows encountered during the survey period and to provide a basis for estimating future conditions of pollution under average or extreme flows such as experience may have shown are likely to occur.

One of the three major sections of the survey will be an extensive compilation of stream flow data for the Ohio and its main tributaries, to be furnished by the U. S. Engineer Corps through the office of the Division Engineer at Cincinnati, Col. E. H. Marks, and under the general and immediate direction of Capt. P. N. Strong and Principal Engineer R. L. Bloor respectively. This work will consist of the analysis of past flow records, showing the average and the 7-day minimum flows during each month of a term of years extending back from 1938, and also the compilation of daily flows, covering the entire period of the present survey, at a series of gaging stations located on the main stem of the Ohio River and at strategic points on its main tributaries. The work of obtaining these data will be aimed especially at showing average and minimum low-water flows, though the entire range of variations will be recorded for the period of the survey.

The very comprehensive data thus made available will be used as the basis of interpreting the laboratory observations in the various streams, both in showing the total quantities of polluting substances per day carried by them at different sampling points and in indicating the degree to which the observations will have to be corrected so as to convert them to terms of normal or minimum flow conditions for the purpose of estimating present or future requirements as to corrective treatment of polluting wastes.

LABORATORY SURVEYS

The first part of the laboratory surveys to be organized is that which deals with observations of pollution conditions in the main stem of the Ohio River, these observations being confined during the current year to the Kanawha-Kentucky River section, which comprises a length of about 280 miles along the middle third of the Ohio River proper. The lower half of this stretch, extending from Dam 33, near Maysville, Ky. to the mouth of the Kentucky River at Carrollton, Ky., will be covered from the laboratory at Cincinnati, where the analytical work will be performed. The upper half, extending from Dam 33 upstream to Dam 25, near Point Pleasant and above the mouth of the Kanawha River, will be covered by a floating laboratory which is being installed on the U. S. Quarterboat "Kiski", which has been loaned for the purpose by the U. S. Engineer Corps as a very substantial contribution to the laboratory work of the Survey.

In the Maysville-Carrollton stretch, the section extending from Dam 36 to Dam 37, in which heavy pollution enters from the Cincinnati Metropolitan District, will be studied more intensively than the remainder of this stretch. In the Point Pleasant-Maysville stretch, the section extending from Huntington to Portsmouth will receive similarly intensive study, as pollution is heavy in this area. In the Cincinnati pool, nine regular sampling stations have been planned, depending on the availability of motor-boat sample collection, and in the Huntington-Ironton section, ten stations have been planned, depending similarly on motor-boat collection. Above and below these two respective sections of the river will be sampling stations more widely spaced and located mostly at Government locks. Arrangements are being made for regular collection of samples by the lock personnel and their transportation to the laboratory by Public Health Service collectors, using automobiles. This collection service has been made possible through the courtesy of the U. S. Engineer Corps, who have offered the excellent facilities of the Government lock personnel and equipment for this purpose.

ROUTINE LABORATORY DETERMINATIONS

The routine schedule of river water examinations will include the following determinations: (1) temperature, (2) turbidity, (3) alkalinity, (4) dissolved oxygen, (5) 5-day biochemical oxygen demand, (6) bacterial count, 24 hours at 37 °C., and (7) coliform group index. Determinations (2) and (3) will be made on samples collected only at one or two key stations from each laboratory. An effort is being made to ascertain whether it will be feasible to substitute gradually a direct-plate count for coliform bacteria instead of the usual enumeration based on fermentation tube tests. In order to establish when the use of modifications of the Standard Winkler procedure will be necessary in making dissolved oxygen determinations, semi-routine determinations of nitrite and also possibly ferrous and ferric iron will be necessary. Special determinations in the river water wil be needed occasionally for pH, CO_2 and acidity, particularly in waters affected by mill drainage and still mill wastes.

In studying the extent and nature of organic sludge deposits above certain dams, arrangements will be made for the regular collection of samples of these deposits by the Government lock personnel at intervals of one or two weeks during periods of low water and following periods of freshets. The primary object of these tests will be to ascertain the extent to which organic deposits are formed in the river during low stages and the extent to which they are removed by scouring action during freshets. In this connection, special attention will be given to sludge deposits formed above the higher permanent dams of which two have been constructed in the upper section of the Ohio. Considerable question exists as to whether deposits are completely removed by freshets from the pool, formed by these higher permanent dams, or whether there will be a tendency for these deposits to accumulate from year to year as the result of incomplete removal. Changes in the character of the deposits from low to high water periods can be gaged by tests of their total B.O.D. or oxidizability and indicators such as the percentage of volatile solids when figured on a dry basis.

Another special field of tests which can be carried out at the Cincinnati and floating laboratories will deal with the character of sewage and certain industrial wastes which may be important factors in the pollution of the river. These tests will necessarily be curtailed to the minimum essential requirements, such as total and volatile suspended matters, settleable solids and B.O.D. Data of this kind will be highly desirable later in considering requirements and possibilities for sewage and industrial wastes treatment at different points. An important problem to be considered in this connection will be the possibilities for treating certain highly organic industrial wastes in combination with sewage.

TRIBUTARY STUDIES

Draining into the Ohio River between the mouths of the Kanawha and Kentucky Rivers are several important tributaries, including the Guyandotte, Big Sandy, Scioto, Little Miami, Licking and Miami Rivers. During the coming year an effort will be made to observe the sanitary condition of these tributaries in their more highly polluted sections, except in the Scioto, where an organized study has been in progress for more than a year past.

At the present writing, two alternative methods of making these observations are being considered. One would consist in operating one or more completely equipped mobile laboratory units, using automobile trailers or trucks, which would travel among the tributaries and collect and examine samples of river water and any important industrial wastes. Mobile units of this kind would be equipped for complete field examinations of samples, including determinations of D.O., B.O.D., 37° bacterial count and coliform group index. The alternative plan would involve using ordinary automobiles equipped with kits for certain tests, such as dissolved oxygen, pH value and alkalinity or acidity, which could be readily made in the field. Samples for more complete examination would be collected and tested at near-by water or sewage laboratories connected with treatment plants. The latter method may be preferable if a sufficient number of plant laboratories are available for the necessary, more complete tests.

Tests of Acid Mine Streams

Owing to the fact that neither of the two base laboratories will be located in the areas most affected by acid mine wastes, it will not be practicable to undertake any extensive observations of streams in these areas during the coming year, except in the Kanawha region, where an effort will be made to develop some systematic field observations based at the floating laboratory "Kiski" in order to provide a groundwork for more extensive observations, it is planned also to set up, under a part-time compensation arrangement, some stream observations based at Morgantown, W.Va., at Norris, Tenn., at Pittsburgh or McKee sport, Pa., and possibly at a fourth point to be selected after consultation with the director of mine-sealing operations in these and other areas. The object of these observations will be to show acid conditions prevailing in some of the larger representative streams draining mining regions, in which fairly extensive mine-sealing operations have been completed and others draining near-by areas in which little or no mine-sealing has thus far been undertaken.

CONCLUSION

The essential difference between the present survey and those which have been made previously by the Public Health Service is that it will be concerned primarily with the more practical measures which may be necessary to correct undesirable conditions of stream pollution, wherever they may be known or found to exist in the Ohio River Basin, whereas the previous surveys have dealt primarily with establishing certain basic relationships and constants which underlie the pollution and natural purification of streams in general. The distinction here involved is that which may be said to exist between a practical situation and its specific remedy and a more general research problem, with its broader implications.

In the present instance the effort thus will be directed mainly to ascertain where actual conditions of pollution are undesirable and, wherever those conditions are observed, to establish a basis for estimating from the known facts concerning sources of pollution, the extent of corrective measures which may be necessary. In some cases the most important consideration involved may be excessive burden on water purification plants. Here the bacteriological and bioligical evidence will assume a primary role. In other cases it may be an undue burden on the oxygen resources of the stream, affecting the maintenance of normal aquatic life, the use of the stream for recreational purposes and perhaps even the general prevalence of nuisance conditions in the adjoining riparian areas. In this latter instance a study of the oxygen balance relationships and their restoration to a favorable status will be a primary consideration. Thus, it is evident that each local area of pollution will have to be studied more or less as an individual problem and the stream observations adjusted accordingly. Although detailed study of many such areas will be inpracticable, in view of the limitations of available time and personnel, it should be possible to locate these areas and to show at least roughly where and to what extent corrective measures will be needed. The Survey will afford an unparalleled proving ground for methods of evaluation which have been developed from previous studies of similar problems. It will be, in a sense, a new pioneering adventure, full of interest and action for those who will participate in it.

Memorandum No. 3

December 29, 1938

Instructions For Routine Bacteriological Examinations

MEMORANDUM NO. 3

December 29, 1938.

INSTRUCTIONS FOR ROUTINE BACTERIOLOGICAL EXAMINATIONS

In conducting the bacteriological examinations for the Ohio River Pollution Survey, the procedure given in Standard Methods for the Examination of Water & Sewage, Eighth Edition, 1936, of the American Public Health Association shall be followed. In cases where choices of equipment, material or procedure are offered, or where interpretation of the procedure given seems desirable, or where a deviation from the Standard procedure is to be made, the considerations which follow shall apply. Any deviations from the Standard procedure will be in the nature of an increased requirement employed as a safety factor.

Sample Bottles: Eight ounce ground-glass stoppered wide-mouthed bottles shall be used.

Pipettes: The 1.0 ml. pipettes shall be graduated in 0.1 ml. and the spacing for the 0.1 ml. portion shall be at least 0.5 inch.

Dilution Bottles: Glass bottles with tight fitting cotton plugs and paper caps will be used, (observing the precautions cited in Standard Methods), unless the newly described rubber stoppers prove satisfactory after trial in the central laboratory at Cincinnati. In using cotton plugged bottles a circular motion of the mixture should be induced before vigorous mixing is started.

Petri Dishes: Side wall of petri dishes shall be 15. mm. high.

Fermentation Tubes: Durham type tubes shall be employed.

Hot Air Sterilization: Two hours at 170°C. shall be the required period and temperature.

Media: Bacto dehydrated culture media as supplied from the central laboratory shall be used for all purposes. Effort has been made to secure sufficient media from one preparation mixture to last throughout the entire investigation.

Steam Sterilization: All media, other than special media which may be employed at times, for which special instructions will be given, shall be sterilized in the autoclave at 15 pounds live steam pressure for 15 minutes, observing the Standard precautions for expelling air, etc.

Dilution Water: Phosphate buffered distilled water, prepared in accordance with instructions in Standard Methods, Part III, Section XII, paragraph 1.2, shall be used. The stock phosphate buffer solution will be supplied from the central laboratory. (See Public Health Reports 48:681, June 16, 1933, Reprint No. 1580, for justification of use of such water for bacteriological examinations.)

Dilutions: The 99.0 ml. portion of dilution water only, will be employed. Intermediate portions in a decimal series of dilutions will be obtained by measuring 0.1 ml. portions directly from the sample or dilution thereof concerned. Experience has shown that, when this precedure is followed with precise technique, employing the 1.0 ml. pipettes required above, the error involved is not as great as when attempts are made to provide and use exactly 9.0 ml. portions of dilution water.

Shaking: In shaking samples or dilutions of samples prior to withdrawal of portions for planting or further dilution, the agitation applied should be vigorous. An addition to the Standard requirement of shaking 25 times, that this be accomplished in 30 seconds when the bottle is moved each time through a space of at least 4 inches, has proved adequate.

Transfer of Portions: In transferring portions of a sample or of dilutions of a sample a fresh sterile pipette of appropriate size should be used for each dilution. On introducing the pipette into the sample for the withdrawal of a portion, the tip shall not be allowed to descend into the sample or dilution thereof more than about 0.5 inch. This precaution is essential as occasionally samples are of such a nature that considerable material will adhere to the outside of the pipette and drain off with the measured portion when it is delivered. Similarly, when portions of a sample or dilution thereof are delivered to dilution bottles the polluted tip of the pipette should not be allowed to touch the neck of the dilution bottle as it enters or as it leaves the bottle. In making the delivery the tip of the pipette shall be brought into contact with the surface of the dilution water, but not allowed to descend into the water. With cotton plugged dilution bottles preliminary mixing of the sample with the dilution water should be accomplished before the cotton plug is wetted by the vigorous agitation specified.

In delivering portions of a sample or dilution thereof to petri dishes the tip of the pipette should be allowed to touch the plate only once in making the delivery. Similarly, in delivering a portion to a broth tube the tip of the pipette shall be placed near the surface of the broth in the tube as delivery is made. This prevents erroneous results due to the adherence of portions of the sample or dilution to the sides of the broth tube above the level of the broth. Organisms cannot demonstrate their presence unless they are actually introduced into the broth.

Delivery from pipettes shall always be made from meniscus to meniscus. Delivery or blow-out pipettes will not be employed and in delivering portions the flow of the liquid shall not be speeded by jerking or blowing. In general, 1.0 ml. pipettes will be employed for measuring 1.0 and 0.1 ml. portions; 2.0 ml. pipettes for 1.0 and 2.0 ml. portions, never for 0.1 ml. portions; and 10.0 ml. pipettes only for portions larger than 1.0 ml.

Examinations: The routine bacteriological examinations to be made on this survey will be (1) total counts of the number of bacteria growing on agar when incubated at 37°C., for 24 hours, and (2) an enumeration of the number of organisms of the coli-aerogenes group of bacteria. Additional special examinations or confirmations which may be instituted as the work progresses will be covered in subsequent memoranda.

Plating: When the portion of sample or dilution thereof is added to the petri dish no more than 15 minutes shall be allowed to elapse before fluid agar at the required temperature is added, thoroughly mixed with the portion and allowed to solidify.

In making plates, two plates containing duplicate portions of the water under examination, which will give colony counts within the prescrived limits, shall be made and also a third plate containing one-tenth or ten times this amount, depending on whether in the judgment of the bacteriologist the count from the duplicate plates will be more than or less than the limiting number. If the number of colonies developing from the samples of a given station are so erratic that the results from the duplicate plates are frequently indeterminate, or if the sample is a new one from an unknown source, then more than one pair of duplicate plates should be made. In making counts of plates a standard illuminated counter will be used.

Supplemental instructions governing the prescribed limits for the permissible number of colonies per plate, the computation of the average bacterial count, and a list of the symbols employed in obtaining and recording results are appended to this memorandum

Tests for Members of the Coli-aerogenes Group: Routine tests as made for this group of bacteria in this study will be limited to the results of the Presumptive and Confirmed Test as given in Standard Methods. The confirmatory test for routine work will be limited to the demonstration of gas production in any amount in 48 hours in the liquid confirmatory medium employed, brilliant green bile (2%) lactose broth. A 3.0 mm. loop will be used for the transfer from lactose broth to the confirmatory medium.

In making inoculations of primary lactose broth tubes for the determination of coli-aerogenes group organisms, three duplicate tubes at each of three decimal dilutions shall be planted. Dilutions for this planting should be selected so that all of the three tubes in the lower dilution will be positive and all tubes in the higher dilution negative, with the results in the intermediate dilution variable. Whenever the results from a given station vary from this ideal to the extent that the results may be indeterminate, i. e. either all positive or all negative, then a fourth dilution should be added to the series of dilutions either higher or lower than the others, depending on the judgment of the bacteriologist. Only tubes of the highest dilution, showing gas formation in lactose broth, shall be transferred for confirmation, except that all tubes negative for gas after 24 hours of incubation, but positive for gas after 48 hours, shall be confirmed. A positive presumptive tube shall always be recorded as positive for the presence of members of the coli-aerogenes group of bacteria, unless confirmation has been tried. That is, the fact that a high dilution tube has failed to confirm cannot be accepted as evidence that a lower dilution gas producing tube, prepared from the same sample, would also fail to confirm. Results obtained will be interpreted in terms of the most probable numbers of coli-aerogenes organisms present, in accordance with the tables given by Hoskins (Public Health Reports, 49:393, March 23, 1934, Reprint No. 1621.) A copy of this reprint will be supplied to each bacteriologist.

Records: When samples arrive in the laboratory pertinent data regarding them shall be entered in a serially numbered log book by the collector and the serial number written on the bottle. The serial number thus assigned to each sample will identify the sample and this number shall appear on each tube, plate or record employed in connection with the sample.

The pertinent data indicated should include adequate information regarding the source of the sample, the time of collection, the name of the collector and the temperature of the water at the time of collection. If any unusual conditions, which might affect either the sample or the envirement at the point of collection, are noted, this information should be entered in the log under "Remarks."

All pertinent data concerning the sample and all results secured in connection with the bacteriological examination of a sample shall be entered on the record cards no erasures shall be made. If an erroneous entry is noted it should be circled and if the correct result is known it should be entered at the nearest available space. The correct result thus entered should be O.K'd by initialing.

RULES GOVERNING THE COMPUTATION OF THE AVERAGE SOLID MEDIUM BACTERIAL COUNT OBTAINED ON PLATES IN USE AT THE U. S. PUBLIC HEALTH SERVICE STREAM POLLUTION INVESTIGATIONS LABORATORY CINCINNATI, OHIO

1. Average for plate counts shall be based on plates which give counts in the range covered by 25 to not more than 400 colonies per plate.

2. When the duplicate plates in a series of three show between 25 and 400 inclusive, colonies per plate, and the third plate less than 25 or more than 400 colonies, the third plate should be omitted from the average unless it falls between the other two.

Examples:

(a)

0.01	c.c.	92	
.01	"	76	
.001	"	6	(omit)
Result		8400	per c.c.

(b)

.1	c.c.	857	(omit)
.01	"	127	
.01	"	156	
Result		14100	per c.c.

(c)

.01	c.c.	92	
.01	"	76	
.001	"	9	(intermediate between duplicate parts)
Result		8600	per c.c.

3. Where the duplicate plates both show too many or too few colonies, only the third plate should be considered in the average result.

Examples:

(a)

0.01	c.c.	847	(omit, too many)
.01	"	732	" " "
.001	"	95	
Result		95000 per c.c.	

(b)

.1	c.c.	65	
.01	"	8	(omit, too few)
.01	"	7	(omit, " ")
Result		650 per c.c.	

4. Where one of the duplicate plates gives an obviously erroneous count it should be disregarded in recording the average result.

Example:

.1	c.c.	68	
.1	"	29	(spreaders) (omit)
.01	"	10	(omit, too few)
Result		680 per c.c.	

5. When one of the duplicate plates comes within the prescribed limits and the other shows too many or too few colonies, both plates must be either included in or excluded from the average as follows, except as indicated under 4: (a) where the average of the two duplicate plates falls within the limits, both shall be included in the average and (b) when the average of the two falls outside

the limits, both shall be excluded.

Examples:

(a)

0.01	c.c.	422)	(average 397, within limit, all plates included in average)
.01	"	372)	
.001	"	39	
Result		395	

(b)

.1	c.c.	255	
.01	"	26)	(average 24, not within limit, both of duplicates excluded)
.01	"	22)	

6. When more than one set of duplicate plates is made, equal authority should be given to each set, providing the number of colonies on the plates falls within the prescribed limits.

EXPLANATION OF SYMBOLS USED IN OBTAINING AND RECORDING BACTERIOLOGICAL RESULTS

For Broth Tubes

0	Indicates no apparent growth.
-	Indicates no gas production.
b	Indicates small amount of gas which remains in spherical form at top of tube.
±	Indicates an amount of gas greater than "b", but less than 10 per cent.
+	Indicates an amount of gas varying from 10 to 49 per cent.
#	Indicates an amount of gas varying from 50 to 100 per cent.

For Confirmatory Plates

0	Indicates sterile plate, no growth.
-	Indicates plate with non-typical
±	Indicates plate with questionable typical growth.
+	Indicates plate with typical colonies.

For Designating Quantities Planted

On record cards portions of sample examined shall be indicated by the decimal system as 10., 1.0, 0.1, 0.01, 0.001, 0.0001, etc. ml. On plates or tubes, where there is a possibility of accidental erasure of some of the figures in handling, the 0.001 ml. portion should be marked 2; the 0.0001, 3; the 0.00001, 4; etc. This system can be easily remembered by noting that the numerals 2, 3, 4, etc. represent the number of ciphers between the decimal point and the unit figure.

Most probable numbers per ml. of sample, planting 3
portions in each of 3 dilutions in geometric series
L = Largest portion M = Middle portion S = Smallest portion

No. of Positive Tubes			3-10 3- 1 3- .1	3-1 3-.1 3-.01	3-.1 3-.01 3-.001	3-.01 3-.001 3-.0001	No. of Positive Tubes			3-10 3-1 3-.1	3-1 3-.1 3-.01	3-.1 3-.01 3-.001	3-.01 3-.001 3-.0001
L	M	S					L	M	S				
0	0	0	--				1	2	0	.11	1.1	11	110
0	0	1	.03	.3	3	30	1	2	1	.15	1.5	15	150
0	0	2	.06	.6	6	60	1	2	2	.20	2.0	20	200
0	0	3	.09	.9	9	90	1	2	3	.24	2.4	24	240
0	1	0	.03	.3	3	30	1	3	0	.16	1.6	16	160
0	1	1	.061	.61	6.1	61	1	3	1	.20	2.0	20	200
0	1	2	.092	.92	9.2	92	1	3	2	.24	2.4	24	240
0	1	3	.12	1.2	12	120	1	3	3	.29	2.9	29	290
0	2	0	.062	.62	6.2	62	2	0	0	.091	.91	9.1	91
0	2	1	.093	.93	9.3	93	2	0	1	.14	1.4	14	140
0	2	2	.12	1.2	12	120	2	0	2	.20	2.0	20	200
0	2	3	.16	1.6	16	160	2	0	3	.26	2.6	26	260
0	3	0	.094	.94	9.4	94	2	1	0	.15	1.5	15	150
0	3	1	.13	1.3	13	130	2	1	1	.20	2.0	20	200
0	3	2	.16	1.6	16	160	2	1	2	.27	2.7	27	270
0	3	3	.19	1.9	19	190	2	1	3	.34	3.4	34	340
1	0	0	.036	.36	3.6	36	2	2	0	.21	2.1	21	210
1	0	1	.072	.72	7.2	72	2	2	1	.28	2.8	28	280
1	0	2	.11	1.1	11	110	2	2	2	.35	3.5	35	350
1	0	3	.15	1.5	15	150	2	2	3	.42	4.2	42	420
1	1	0	.073	.73	7.3	73	2	3	0	.29	2.9	29	290
1	1	1	.11	1.1	11	110	2	3	1	.36	3.6	36	360
1	1	2	.15	1.5	15	150	2	3	2	.44	4.4	44	440
1	1	3	.19	1.9	19	190	2	3	3	.53	5.3	53	530

Sheet No. 2

No. of Positive Tubes			3-10 3- 1 3- .1	3-1 3-.1 3-.01	3-.1 3-.01 3-.001	3-.01 3-.001 3-.0001
L	M	S				
3	0	0	.23	2.3	23	230
3	0	1	.39	3.9	39	390
3	0	2	.64	6.4	64	640
3	0	3	.95	9.5	95	950
3	1	0	.43	4.3	43	430
3	1	1	.75	7.5	75	750
3	1	2	1.20	12.0	120	1200
3	1	3	1.60	16.0	160	1600
3	2	0	.93	9.3	93	930
3	2	1	1.50	15.0	150	1500
3	2	2	2.10	21.0	210	2100
3	2	3	2.90	29.0	290	2900
3	3	0	2.40	24.0	240	2400
3	3	1	4.60	46.0	460	4600
3	3	2	11.00	110.0	1100	11000
3	3	3	- -	- -	- -	- -

Memorandum No. 4

January 6, 1939

Outline of Chemical Methods

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OUTLINE OF CHEMICAL METHODS

The routine chemical analysis to be made on water samples during the Ohio River Pollution Survey will include the following determinations: Turbidity, alkalinity (and/or acidity, if indicated), total and volatile suspended solids, pH, nitrite nitrogen, dissolved oxygen and 5-day biochemical oxygen demand. The dissolved oxygen shall be determined on individual samples collected at each point of every sampling section or station. Biochemical oxygen demand shall be determined on the total composite of the left, center and right point samples at each sampling station. If a sample from only one point is collected at any sampling station, the B.O.D. must be determined on it.

Nitrite determinations are to be made to determine what method must be used in making the dissolved oxygen determinations. If the dissolved oxygen determinations are to be made in the field to the point of titration (at points most distant from the central laboratory) then the nitrite determination must also be made in the field. In all samples where .05 p.p.m. or more nitrite is found, a procedure for destroying nitrite must be used before dissolved oxygen is determined. If less than .05 p.p.m. of nitrite is found, the unmodified Winkler method may be employed. If, after a period of daily nitrite examinations, it is found that most sampling points are likely to contain nitrite above .05 p.p.m. at times, it will be best to adopt the corrective procedure in all cases and to eliminate the nitrite determinations.

Turbidity, alkalinity, total and volatile suspended solids and pH determinations will be made only on samples from special designated stations. The water sample for these determinations may be poured from the sampling can into a wide mouth 32 oz. bottle and transported to the laboratory. At stations where three points on a section of the river are being sampled, this bottle shall be poured about one-third full at each point from a measure, provided for the purpose, full of water taken from the sample collector by dipping. In all of these determinations the methods described in Standard Methods for the Analysis of Water and Sewage, Eighth Edition, will be used as far as possible. A brief description of these procedures, with detailed instructions in any cases of deviation from these methods, follows:

Turbidity

The square 32 ounce bottles containing the special samples may be compared with standards of silica suspensions prepared in the same kind of bottles for turbidities of 25 p.p.m. and less. The standard candle turbidimeter shall be used to determine turbidities of over 25 p.p.m., the vanishing point taken as the depth of sample at which the image of the standard candle flame first becomes evenly diffused over the entire cross section of the tube.

Hydrion Concentrations (pH)

The pH determination shall be made electrometrically using the glass electrode. (A Leeds and Northrup Universal pH indicator will be supplied to the boat for this purpose and the L. & N. meter using the old, larger glass electrodes will be available at this Station.) When the calomel and glass electrodes have been prepared and the meter has been assembled, the following precautions should be observed in making a pH reading. First, the vessel which is to contain the sample for measurement must be thoroughly cleaned. It should be rinsed once with distilled water and once with the sample to be examined before it is filled with sample. The temperature of the sample should be taken at this point and the temperature compensation dial on the meter adjusted properly. The dry cells or other source of E.M.F. are then balanced against the standard cell of the meter by intermittently pressing the key marked S.C. and adjusting the variable resistance until no deflection is obtained on the galvanometer. The pH of the sample may then be determined by intermittently pressing the key marked pH and adjusting the pH scale until the galvanometer shows no deflection. When no deflection of the galvanometer is obtained by pressing the pH key the scale reading indicates directly the pH of the sample. If the dry cells cannot be balanced against the standard cell, the dry cells are run down and must be replaced before a pH reading can be obtained. On any sample in which the pH is found to be 5.1 or less, a determination for acidity is indicated.*

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* At points in the upper stream where acidity determinations are necessary, this determination should be made by titration of hot and cold portions of sample with phenolphthalein and by titration of a cold portion using methyl red as an indicator. Methyl red with a color change at a pH of 5.1 should not be substituted for methyl orange in the alkalinity determination. Neither should methyl orange with a color change at pH 4.0 be substituted for methyl red in the acidity determination.

Alkalinity

The alkalinity should be determined oh a portion of the special composite sample from the 32 ounce bottle. The Standard Method should be used, employing N/50 $H_2 SO_4$ and methyl orange indicator.

Total and Volatile Suspended Solids

The suspended solids should be determined by filtering 50 to 100 ml. of the special sample, with suction, through a Gooch crucible which has been prepared with an asbestos mat, dried, ignited and weighed. After filtration the crucibles are dried at 103 to 105° C. for 2-1/2 hours, celled in a desiccator and weighed. The crucibles are to be ignited at 600° C. for 10 to 15 minutes in the muffle furnace at the main laboratory and may be heated at dull red heat with a Meeker burner on the boat in determining the volatile suspended solids.

Nitrite Nitrogen

This test, employing the Standard Method, is to be carried out in the field or immediately after the samples arrive at the laboratory, but in any case before the D.O. determination is started. For the field test, our sample collector should be provided with the reagents for making this test (sulfamilic acid solution and anaphthylamine acetate solution) together with the necessary standard solution, 2-500 ml. bottles of distilled water, a number of pipettes and two 100 ml. low form Nessler tubes. All of this material, along with the reagents for making the D.O. determinations will be carried in a special kit provided for that purpose. The nitrite test should be made on the composite sample at each sampling station as soon as the sample is collected. This test shall be directed to determine whether or not nitrite is present rather than to determine the exact amount present. Consequently, only one standard of .05 p.p.m. will be required and it will be necessary only to determine whether or not the nitrite nitrogen content is less than .05 p.p.m.

Dissolved Oxygen

Because of the probability of encountering the various interfering substances in any investigation of the scope of Ohio River pollution survey, it is impossible to select one procedure for dissolved oxygen that will be applicable in all cases. Other interfering substances besides nitrite, such as ferrous and ferric salts, organic matter and sulfite wastes, may be encountered in samples from some points and the chemist must be on the alert for evidences of these materials so that the best corrective procedure for any particular material may be applied. On all upper river sampling points the chemist should make ferrous and total iron determinations before the method of determining dissolved oxygen is selected.

The following procedures are all applicable under certain circumstances. These are listed in the order of their simplicity and the special conditions under which they should or should not be applied:

(1) Regular Winkler Method (See pages 144-145 Standard Methods) This method should be applied on samples from all points of the main stream which contain less than .05 p.p.m. of nitrite, 1.0 p.p.m. of ferrous iron and 10 p.p.m. of total iron. On all of the lower river points the iron content will probably be lower than the above limits so that the nitrite test will determine whether the regular Winkler method should be used.

(2) Short Winkler Method This method should be applied to samples containing mud or sludge where the limits mentioned above for nitrite and iron are met, but where a large amount of interfering organic matter is present. Consequently, it will rarely be necessary to use this method on Ohio River samples.

(3) The Alsterberg Sodium Azide Procedure This procedure should be applied on Ohio River samples where the iron content meets the limits stated under (1), but where more than .05 p.p.m. of nitrite nitrogen is found. The detailed procedure for this method is given in the Analytical Edition of the Journal of Industrial and Engineering Chemistry, Vol. 10, No. 12, page 701 (1938).

(4) The Rideal Stewart Modification should be used on any Ohio River samples which contain appreciable (1.0 p.p.m. or more) amounts of ferrous iron. As each p.p.m. of ferrous iron would produce an apparent loss of .14 p.p.m. of dissolved oxygen, one part is taken as the upper limit of tolerance for the ordinary Winkler or Alsterberg procedure. If more than 1.0 p.p.m. is present this should be oxidized by the acid permanganate

following the Rideal Stewart procedure (pages 147-148 Standard Methods).

(5) The Alkaline Hypochlorite Modification This method may be necessary at sampling points where considerable quantities of paper mill wastes are encountered.

5-day Biochemical Oxygen Demand

The 5-day biochemical oxygen demand determination shall be made according to the standard method, except in the following details of procedure:

I - The phosphate buffered formula C water(1) shall be used wherever dilution of samples is required. The four solutions required to make up this dilution water are:

(A) Ferric chloride - 0.27 g. $Fe\,Cl_3\;.6\,H_2O$ per liter

(B) Calcium chloride - 18.3 g. $Ca\,Cl_2\;.4\,H_2O$ per liter

(C) Magnesium sulphate - 9.9 g. $Mg\,SO_4\;.7\,H_2O$ per liter

(D) Phosphate buffer - 34.04 g. $KH_2\,PO_4$ and 175 ml. of N/1 NaOH per liter

The following amounts of each of these solutions are added to distilled water, free of copper, to prepare the dilution water:

(A) 0.5 ml. per 1. or 8.0 ml. per 16 1. carboy

(B) 2.5 ml. " 1. "40.0 ml. " 16 1. "

(C) 2.5 ml. " 1. "40.0 ml. " 16 1. "

(D) 1.25 ml. " 1. "20.0 ml. " 16 1. "

The bicarbonate dilution water of Standard Methods shall not be used.

II -The incubation bottles should be 250 ml. capacity, with ground-glass stoppers, cleansed with concentrated sulphuric acid, carefully rinsed and dried before use. Water seals may be dispensed with except during the summer months when undiluted river water with an initial temperature higher than 20°C. is to be incubated. On any special B.O.D. work in which a series of bottles are incubated for long periods it is desirable to water seal the bottles during incubation.

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(1) Theriault, E. J., McNamee, P. D., and Butterfield, C. T. Experimental Studies of Natural Purification in Polluted Waters. V. Selection of Dilution Water for Use in Oxygen Demand Tests. Public Health Reports 46, 1084-116(1931).

III- On any sample in which the pH is found to be below 6.0 it will be necessary to adjust the pH to 7.0 to 7.2 with N/10 NaOH before incubation for biochemical oxygen demand. After the pH adjustment has been made, the sample should be reseeded with river water at a normal pH. About 20 ml. of river water per liter of sample or about 5 ml. for an individual D.O. bottle should be used for seeding.

IV- Where it is necessary to dilute the sample before incubation, the dilution technic employed should not reaerate the diluted sample. This procedure is chosen in order that a calculated initial dissolved oxygen content of the diluted sample may be made from the known dissolved oxygen content of the sample and the dilution water, if it is desired. For this reason, the dissolved oxygen content of the dilution water used with any sample must always be determined and recorded on the sample card. It will probably be found that 50 percent dilution will usually suffice on Ohio River samples that require dilution. In rare instances it may be necessary to make a 33-1/3% or even a 25% dilution of the sample. When the dilution factor has been decided it should be recorded and the proper volume of sample should be siphoned into a one liter cylinder. Dilution water is then siphoned into the cylinder to the one liter graduation, being delivered below the surface of the liquid, and the dilution is thoroughly mixed without aeration with the special stirring device. After mixing, the sample is immediately siphoned into three properly labeled 250 ml. B.O.D. bottles. If the diluted sample contains considerable settleable suspended matter, the stirring should be continued while the sample is being siphoned into bottles. The dissolved oxygen content of one bottle is determined at once. The other bottles are incubated at 20° C. for five days and the final dissolved oxygen content is then determined. If the amount of other work required makes it necessary, the check bottle, after incubation, may be eliminated. In making the dissolved oxygen determinations for the B.O.D. test the sodium azide modification of the Winkler procedure should be used when necessary, in order to eliminate interference due to nitrites that may be formed during incubation.

V - If the initial mean dissolved oxygen content of the water at any sampling section is over 9.5 p.p.m. the oxygen content of the water shall be reduced before the sample is incubated for the B.O.D. determination. In such cases the water sample for the B.O.D. composite is poured into a bottle about twice the size necessary to hold it. The stoppered bottle is then carefully warmed in a water bath until the temperature of the sample reaches 20 to 30° C. The bottle is then vigorously shaken for several minutes to remove the excess oxygen. After shaking the sample is cooled to 20° C. and siphoned into 250 ml. B.O.D. bottles. The initial dissolved oxygen content is determined on one of these and the others are incubated at 20° C.

Memorandum No. 5

January 12, 1939

Instructions Concerning The Use Of Bacto-Brilliant Green Lactose Bile Agar For The Enumeration of Organisms Of The Coli-Aerogenes Group of Bacteria

Memorandum No. 5

January 12, 1939

INSTRUCTIONS CONCERNING THE USE OF BACTO-BRILLIANT GREEN LACTOSE BILE AGAR FOR THE ENUMERATION OF ORGANISMS OF THE COLI-AEROGENES GROUP OF BACTERIA

Comparative results on the enumeration of coli-aerogenes group bacteria in river water obtained, (1) by the Standard procedure using lactose broth as a primary medium, with three duplicate tubes at each dilution in three or more decimal dilutions of a sample, and (2) by plate counts from the same samples using a differential plating medium, Bacto-Brilliant Green Lactose Bile Agar, have indicated a very high degree of correlation.

As results by the direct plating method are much easier to obtain and are not subject to the errors common to the dilution method, this procedure will be followed for the enumeration of members of the coli-aerogenes group of bacteria for samples from certain sampling points in the Ohio River Pollution Survey, either in addition to the determination as made by the Standard procedure, or with the exclusion of the determination by the Standard procedure. The sampling points from which samples will be examined for coli-aerogenes group bacteria by the direct plating method and instructions as to whether this method shall be used in addition to, or to the exclusion of the Standard procedure, will be set forth in subsequent communications.

Procedure to be followed: In general the procedure for making plate counts after 24 hours incubation at 37° C., as given in Standard Methods and as interpreted in Memorandum No. 3 (December 29, 1938), will be followed. Certain detailed items of technique, which apply to this direct plating procedure, will now be given.

Preparation of Medium: All media for this study will be prepared from dehydrated Bacto-Brilliant Green Lactose Bile Agar (Serial No. 36793) in accordance with the instructions given, (Reference Noble & Tonney, Jour. A.W.W.A. 27:108: 1935). Single strength medium, which is to be used for planting 2. ml. or less portions, is prepared by dissolving with heat 20.6 grams of dehydrated powder in 1000. ml. of distilled water. Care must be exercised to prevent the powder burning on the bottom of the vessel before it is entirely dissolved. As soon as the powder is completely dissolved, mix thoroughly and distribute into agar bottles, putting 50 to 150 ml. in each bottle, depending on the daily requirement of the particular laboratory. Cotton plug, paper cap and mark the bottles as for Standard agar. Sterilize at 15 lbs. live steam pressure for 15 minutes. (The time requirement of 20 minutes cited on the container presupposes the availability of live steam from pressure lines. With our conditions, requiring a longer period for the development of 15 lbs. of steam pressure when the steam is generated in the autoclave, a 15 minute interval, after the correct pressure has been reached, has been found sufficient). Medium, thus sterilized, will be stored in the refrigerator until required for use. This period of storage should not exceed 10 to 14 days as it will deteriorate after this period.

Planting: The general instructions referred to will be followed. Portions of samples, (diluted if necessary), should be planted of such volume that the counts obtained for members of the coli-aerogenes group will be within the range of 25 to 400 colonies per plate. The ideal range of colonies per plate is from 50 to 150. As many pairs of duplicate plates as necessary, to obtain counts within the permissible limits, may be planted, but it should be possible after experience with the particular samples under investigation to limit this number to two pairs.

Sufficient agar at a temperature of 40 to 41° C. should be poured into the plates to provide for a uniform depth of agar of approximately 0.3 cm. in the finished plate. This will require 15 to 20 ml. of agar per plate depending on the diameter of the particular petri dishes employed.

Incubation and Counting: Plates, thus made, will be incubated at 37° C. for 16 to 18 hours before counting. They should be counted during the interval between 16 and 18 hours of incubation as prior to this period all colonies may not be characteristically visible and after this period extraneous colonies, whose appearance may be confusing, may appear. In counting the colonies, an illuminated counter provided with a white or silver-gray background should be employed.

As the bacterial content of a sample may change quite rapidly, the samples should be planted as soon as they arrive in the laboratory. Where comparative coli-aerogenes group enumerations are being made with the results obtained (1) by the direct plating method and (2) by the Standard lactose broth enrichment method, plantings should be made for both enumerations at the same time. These requirements, if the sample should arrive in the laboratory during the morning hours, say 10 A.M., would mean that the 16-18 hour period of incubation would be fulfilled at an inopportune time, namely at 2 to 4 A.M. of the following day. To provide for these difficulties and at the same time meet the incubation requirements, the Brilliant Green Lactose Bile Agar plates may be placed in the refrigerator immediately after they are planted and solidified. At this stage the bacterial cells from the sample are held fixed in the agar and growth, as thought of in terms of 37° C. incubation, is stopped. Plates held thus in the refrigerator may be placed in the 37° C. incubator at 3 P.M. and the 18-hour incubation period will then be complete at 9 A.M. the next day. For plates that have been chilled in this manner in the refrigerator, approximately an 18-hour period of incubation should probably be allowed, rather than 16-18, as it will take approximately an hour for the cold plates to get to incubation temperature. Experience with counting and with the particular refrigerator available will indicate the proper time interval to the bacteriologist.

Recognition and Identification of Colonies: While colonies formed by members of the coli-aerogenes group of bacteria in this medium do have a characteristic morphology and a black to red color, familiarity with their appearance can be obtained more definitely with visual inspection and testing than by attempts at verbal description. This knowledge should be obtained in two ways: (1) by planting pure cultures of coli-aerogenes bacteria, in appropriate dilution, and observing carefully the appearance of the colonies; and (2) by picking colonies on plates made from natural samples for confirmation. To carry out this latter procedure, all of the colonies counted from a given area on a plate (10 is an appropriate number) should be picked to brilliant green bile lactose broth for partial confirmation. The production of gas in any amount in this broth, after 24 to 48 hours of incubation at 37° C., shall be accepted as evidence that the colony picked contained some member of the coli-aerogenes group of bacteria.

To insure fairness in the identification and to avoid the selection of particular types of colonies, an area should be circumscribed on the bottom of the plate enclosing about 10 colonies which have been counted and all of the colonies enclosed within this area which have been included in the coli-aerogenes count should be picked. This procedure should be followed with each sample and records kept of the results until the bacteriologist has established a high degree of accuracy, at least 90 per cent, in the selection of the colonies counted.

Recording of Results: The rules given for the recording and averaging of results in Memorandum No. 3 shall be applied to results obtained with this special medium.

Memorandum No. 8

March 1939

Collection And Examination of Biological Samples

and

Supplement on Points To Be Covered

13-A

Memorandum No. 8

March, 1939

COLLECTION AND EXAMINATION OF BIOLOGICAL SAMPLES

In the following memorandum, detailed directions are given concerning the collection and biological examination of river water and bottom sediment samples. It is desirable, in this connection, to note here very briefly, the broad objectives of the biological examinations in a survey of the general character represented by the one now in progress in the Ohio River Basin.

These objectives may be stated as follows, having in mind the essentially practical nature of the present survey:

1. A study of the distribution of pollution and non-pollutional organisms in the river water and in the bottom sediments, both in the more highly polluted sections of the river and in those affected more remotely by pollution. In this connection, reference may be made to the results of previous observations in the Ohio and Illinois Rivers.

2. A survey of stream sections affected by certain type of industrial pollution, such as acid mine, tannery, paper pulp, soap, chemical and other similar wastes, with a view to estimating the extent to which these waters may interfere with the normal distribution and functions of organisms which are concerned in natural purification of the stream.

3. A special study of the occurrence of organisms in the river causing tastes and odors in water supplies, or interference with the economical purification of water supplies. This would include a consideration of pollution and other conditions in the river which might have a bearing on the prevalence of taste-producing or filter-clogging organisms.

The present survey differs very markedly from previous studies of the Ohio River in that it is not primarily a research project. It is concerned with locating seriously polluted stretches of the Ohio and its tributaries and with obtaining the observational data necessary to estimating the nature and extent of corrective measures which will be required in each situation for restoring a stream to a reasonable degree of cleanliness, having in mind the more essential water uses of the particular stream. For this reason it is not proposed to undertake establishing any extensive array of new facts or principles bearing on the fundamental nature of stream pollution and purification phenomena, but rather to utilize those facts which are already know as the basis of evaluating the specific problems of stream pollution which now exist in the Ohio River and its tributaries. This general viewpoint may be said to be a guidepost for all of the laboratory studies connected with the present survey, including the biological studies.

A. Plankton Samples

For the present, the regular bacteriological samples will serve as plankton samples also.

On arrival of these bacteriological samples at the laboratory, or at the laboratory boat "Kiski", the biologist, if present, will arrange, with cooperation of the officer in charge, to secure a portion (about 100 cc or more) of the sample.

If the biologist is located elsewhere temporarily, (perhaps on field duty) the person in charge of the samples, or some person designated by the Director of the Laboratory, should procure the plankton samples from the bacteriological samples as follows:

1. Compositing of samples, if necessary* should be done as soon as possible after arrival of the samples.

2. One hundred cc or more of the composited water is to be dosed with 3 cc of formalin, labeled stating station and date, and set aside for the biologist when he shall arrive.

3. In any case, 100 cc is the minimum amount required for a plankton sample. If the biologist is present, he will centrifuge the 100 cc sample at once, and will examine the unkilled catch. In case of 2 or more samples requiring attention at the same time, the unkilled 100 cc sample is to be placed in the ice box until it can be centrifuged.

B. Bottom Sediments (To be collected once a month at selected stations.)

Equipment Required:

One small dredge, or a mushroom-shaped mud scoop, with 50 or 60 foot line attached, 1 small curved garden trowel or stout dipper, strips of tough white paper about 1 x 5 inches (for labels) and mason quart jars, one for each sample, with about 80 cc of formalin in the jar.

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* Necessary only in case of E. C. & W. samples.

Procedure:

(1) Throw the scoop out, harpoon fashion, as far as possible from the boat. When the scoop has reached bottom drag it 5 or 6 feet to secure a bottom sample. Pull the scoop and contents aboard.

(2) With trowel or dipper select portions of the mud in the scoop representative of the entire catch and drop these selections in the jar. (Guard against splashing formalin in your face.) Make the jar three-fourths full, or more.

(3) Using a soft lead pencil (do not use indelible), write the station, the location in channel (E. C. or W.) and the date, and drop this label in the jar. Deliver the sample to the biologist, or to the man who transfers samples to the laboratory.

(4) If the sampler brings up pebbles, rubbish, fungus-like masses attached to stones or sticks, etc. include a fair proportion of these things in the jar sample.

(5) Bottom sediments are sometimes swept away by flood conditions. The first try may fail to get a bottom sample. Try another portion of the channel. Get the sample if possible. This is important.

(6) Discard mud remaining in sampler, rinse this, coil line, and have everything ready for taking the next sample.

C. Examination of Samples (Plankton)

1. Concentration

Mix the sample thoroughly by gentle agitation. Place a measured amount, 100 cc if possible, in centrifuge tubes, and centrifuge for five (5) minutes at high speed (about 3500 r.p.m.). Pour off excess water and remove the catch (by catch is meant the concentrated contents of the entire sample) from the centrifuge tubes to a bottle or vial already provided with a label stating the station, the date, and the concentration of the catch. Get all of the catch from the tubes by rinsing with a half cc of clean water, adding this rinsing to the catch already in the vial. The total amount of the catch is now measured, using, for convenience, a 10 cc delivery pipette. If (a) the amount is, for example, 4½ cc. sufficient water should be added to eliminate the fraction, thus making 5 cc. (b) If the amount be 8 or 9 cc. it is advisable to add enough water to bring the total volume up to 10 cc. This data should be added to the label: "100 cc to 5 cc" or "100 cc to 10 cc." This statement of concentration means that by counting all the organisms in all of the catch, we thus ascertain the total content of the 100 cc sample of water. Similarly, if we count the organisms in 1 cc of the catch in (a) above, we thus ascertain the content of one-fifth of the original water, or 20 cc.

2. The Count

The Counting-Cell Method: The microscopical examination or counting is accomplished as follows, using the Sedgwick-Rafter counting cell, which holds 1 cc and has a depth of exactly 1 millimeter:

(a) Thoroughly, but gently, mix the entire catch.

(b) Using a graduate pipette which does not have a fine tip (this may be broken or filed off) deliver 1 cc of the mixed catch to the cell.

(c) Affix the glass cover by a side-ways motion of this cover, as suggested by (a) Whipple.

(d) With a low power microscope (about 20 or 30 diameter) examine the entire cell, moving it back and forth across the stage and inspecting every portion of the 20 x 50 millimeter area thus presented. This is a search for any relatively large organisms, such as caustacea, rotifers, small worms and certain plant forms. Tabulate the number and kinds of these larger organisms, if any are present, in the "Survey" (or "Once-over") column of the plankton counting sheet. If catch (a) is used, this large organisms count is really the content of 20 cc of the original water. There may be a dozen organisms, or maybe only 3 or 4, or perhaps none at all.

(a) Microscopy of Drinking Water, Revision by Fair and Whipple, page 96.

(e) The plankton counting sheet recommended by Whipple, provides not only for this large organisms content, but also for a representative count of the smallest organisms, which are usually very much more numerous than the large ones. For this small organism count, either 5 or 10 fields are selected in representative positions of this counting cell, and the organisms in these fields are counted under a higher magnification, usually 16 mm. objective and 10 x ocular. This ocular is provided with a Whipple Ocular michrometer disk, and the tube-length of the microscope is adjusted by use of a stage michrometer so that the largest square on the disk exactly subtends 1 square millimeter on the stage. This square millimeter is the "field" to be counted, and since the counting cell is exactly 1 millimeter in depth, we are counting, on the floor of the cell, the organisms that represent 1 cubic millimeter of the catch from the original plankton sample. Five fields therefore represent 5/1000 or 1/200 of the contents of the counting cell, this cell being 20 millimeters by 50 millimeters and having an area of 1000 square millimeters. Ten fields counted represent 10/1000 or 1/100 of the contents of the counting cell, and so on, whatever numbers of fields may be counted. If, for instance, we have in the 5 representative fields counted:

(1) 20 of Organism A
10 " " B
15 " " C

we know that these totals are only 1/200 of what are actually in the entire cell, so multiplying by 200 we have:

(2) 4000 of Organism A
2000 " " B
3000 " " C

These largest numbers represent the actual total content of the counting cell.

Now, because this "catch" in the counting cell is a concentration, we must take this fact into consideration before we can state the result of our count in terms of the original sample of water. Since we concentrated the 100 cc sample to 5 cc (in the case of (a)) one cc of the concentrated "catch" must therefore contain the organisms content of 20 cc of the original water. In other words, the totals stated in (2) are twenty times as great as the actual content of the original water. To find this latter value, therefore, we divide these items by 20, with results as follows:

(3) 200 of Organism A
100 " " B
150 " " C

For convenience, a formula involving the preceding relative values is often used. It is as follows:

$$\frac{\text{Cubic millimeters in 1 cc of the catch (in the cell)}}{\text{Cubic millimeters (fields) examined}} \times \frac{\text{cc of concentrated "catch" obtained}}{\text{cc of original sample of water used}} = \text{Factor}$$

Substituting values in the above formula:

$$\frac{1000}{5} \times \frac{5}{100} = \text{Factor } (= 10)$$

This "factor" is the number by which we must multiply our 5-field count data in order to express these values in terms of the actual content of the original water.

The Cover-glass Method: Lackey's method (a) of counting a definite portion of the catch by using several cover-slip mounts instead of the old-time counting cell has the unquestioned advantage of enabling the operator to switch instantly to the high power when the low power is found insufficient to identify the organism. Such occasions may be frequent, and the cover-glass method may be the preferable method, particularly if the large majority of the plankton organisms are very small. The large forms, however, should not be overlooked, although they are usually few in comparison in many cases, - fewer than 1 per ml. (or cc.). Quantitatively considered, however, these few large organisms may nearly equal, or possibly overbalance, the larger numbers of very small organisms. One Rotifer tardus equals approximately 10 Codonella, or 80 Halteria, in volume. An ideal procedure would seem to be a combination of (1) the cover-glass method, because of its great advantage of identification by use of the high power, with (2) a rapid count, or inspection, of a larger quantity of the plankton catch either in a 1 cc counting cell or by inspecting, under low power, all of the area of at least 10 cover-slip mounts, which would be the equivalent of about one-half the 1 cc counting cell. This procedure would at once reveal the presence-or the absence-of the larger organisms and their relative abundance.

(a) Public Health Reports 53: No. 47, Nov. 25, 1938. Manipulation and Counting of River Plankton. By J. B. Lackey.

Lackey's cover-glass method (see (a)) is essentially as follows:

1. The centrifuged catch is mixed, and measured in terms of drops by using a pipette, so that 1 drop of catch bears a definite relationship to the amount of sample centrifuged. For example, if a 100 ml. sample, centrifuged, yields 76 drops of catch, enough of the decanted portion (in this case 24 drops) is added to make a total of 100 drops. This makes 1 drop of the catch equal 1 ml. of the original.sample.

2. To count, place 1 drop of the catch on the center of a slide and cover with a No. 1 25 mm. cover glass (preferably square).

3. Using the low-power objective, count two paths entirely across the spread-out drop, one path horizontal, the other vertical, the two paths crossing at or near the center of the drop.

4. Repeat (3) with several other drops, perhaps ten or more, until the total or combined width of the twenty or more paths counted (2 paths per drop) is equivalent to the total diameter of the square cover-glass. In other words, the equivalent of 1 entire drop has been counted, and this completed count represents the organism content of 1 ml. of water of the original sample. In an unkilled catch, some of the organisms will quickly migrate to the edge of the cover-glass, seeking oxygen. Others will die, becoming motionless and perhaps difficult to detect. Still others will migrate toward the lighted edge or portion of the drop. By counting only two paths, then repeating this with a fresh mount, and

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(a) Public Health Reports 53; No. 47, Nov. 25, 1938. Manipulation and Counting of River Plankton. By J. B. Lackey.

so on (see 4) the above difficulties are minimized or avoided. The low-power examination will not take into account some of the small but significant organisms. Hence the same procedure is followed with the high dry (10 or 12.5 x oculars, 40-45 x objective) combination, as with the low. However, one cover-glass comprises about 80 paths at a magnification of ca. 500 diameters, so only a few paths (6, 8 or 10) are counted instead of a whole drop. This makes it necessary to use a factor for estimating the numbers per ml. but if the procedure is constant, this factor and any error it introduces will be a constant. Furthermore, the entire catch from 100 ml. may usually be encompassed in 50 or even 25 drops.

5. The entire area of each drop may be quickly inspected with the low-power in order to note the possible presence of any large organisms, such as worms, rotifers and crustacea (b). If present in 10 ml. of the original water sample, they will usually show up in the examination of the entire area of such a number of one-drop cover-glass mounts of plankton catch as are required to represent 10 ml. of the original water. Large organisms as Paramecium, Aeolosoma or Rotifer may be counted by using 2 x or 5 x oculars with the 10 x objective, thus permitting a very quick examination of an entire drop.

D. Bottom Sediments

Pending acquisition of a suitable dredge by which quantitative areal samples of bottom mud may be secured, the old-time mushroom-shaped scoop, formerly used on the Potomac, the Ohio and the Illinois Rivers, may be used to secure a qualitative sample. This sample will furnish valuable information as to the make-up and the apparent sanitary status of the stream bottom, and the population of this mud (usually worms, mollusks, insect larvae and similar forms) will give further information along the same general lines.

The present plans call for one bottom sample per month from such locations as are thought to be representative. These locations will usually coincide with some of the plankton sampling stations, but may not be so numerous. Moreover, one point, instead of three (E. C. & W.) will usually be sufficient.

(b) See Public Health Reports, Nov. 18, 1938. Protozoan Plankton as Indicators of Pollution. J. B. Lackey.

This one point, at a given station, should be one where mud is available. It frequently happens that soft bottom (sediment) is available at only one of the E. C. & W. positions.

Collection of Sediments - See "B - Bottom Sediments"

Laboratory procedure with the mud samples

Equipment needed:

One.....2-liter mug-like dish, with stout handle (a 2-liter "Miner's cup" is ideal. Also half of a double-boiler is convenient).

Two or three. Moist chambers, large size (about 10 in. diameter)

One.....7-inch square of 25-30 mesh brass screen (or cloth) with corners well rounded off.

1 pair deep-jar forceps.

Proceed as follows:

1. Partly fill a moist chamber with clean water.
2. On the outside of the mason jar containing the mud sample, mark, with grease pencil, the upper level of the mud.
3. Empty the sample or part of it from the jar into the 2-liter "miner's cup" or other container. Record color, pastiness, odor, presence of pebbles, etc.
4. Introduce a partial fold extending from one side of the square of brass cloth, about to the center (not beyond) of this square. Press this partial fold over, and grasp the now dished screen with thumb and finger at this triangular area where there are now 3 thicknesses of the screen. Thoroughly wet this screen on both sides.
5. Run 100 cc or more tap water into the miner's cup or other vessel containing the mud sample. Agitate by a vigorous rotary motion and at once pour the suspension through the sieve or screen, which you are holding firmly with thumb and finger of the other hand.

6. Run more tap water into the container, agitate as before, and pour through sieve.

7. When some "screenings" have accumulated, invert the dished screen upon the surface of the clean water in the moist chamber, - slosh screen gently up and down, washing the catch from the now under side. It is convenient, at this point, to reverse the "dished" condition of the screen.

8. Proceed as in 5, 6 and 7 above, until all of the mud sample has been washed through the screen. Pebbles, sand, etc. will not wash through, and may be discarded after inspection by the biologist.

9. Run tap water into the jar to the grease-pencil mark (see 2). Measure this water. This is the amount of your mud sample. Record on your examination sheet this amount to the nearest multiple of 5, like 875 cc, 760 cc, 985 cc, 800 cc, etc.

10. Examine the catch in the moist chamber. Count or estimate the number of worms, mollusks, insect larvae, or other organisms. Note the presence of kinds of attached growths, such as fungi, algae, stalked ciliates, bryozoa, and the like. Inspect the catch for the presence of capsules, indicating the propagation of tubificid worms.

    Note any unusual abundance of detritus of any kind, such as leaf fragments, straw or chaff, bits of paper, fecal pellets of worms or insect, molts of insects, fibers apparently from a paper mill, saw dust, ect.

    If pebbles, or sand are present in considerable amount, the relative proportion of such material should be noted. For instance, a sample measuring a total of 850 cc may consist of about 200 cc of pebbles, 150 cc of sand and only 500 cc of actual mud. As a rule, these materials (pebbles and sand) need not be introduced into the moist chamber at all (see 8) but if present in considerable amount in the sample this approximate amount should be recorded.

11. The record

    a. Location, date and amount of sample.
    b. Color, condition and odor.

c. Predominance, or relative abundance of plant fragments, detritus, wastes, sand peebles, etc.

d. Population:
    - Worms (tubificid, naid, etc.)
    - Mollusks (bivalves and univalves)
    - Insect larvae (Chironomids, Mayflies, Caddis flies, etc.)
    - Attached forms such as bryozoa, stentors, stalked ciliates, etc.
    - Attached plant forms (diatoms fungi, algae)
    - Detached plant forms (algae, fungi, submerged plants of the higher types, etc.)

12. Final statement of population density should be in terms of 1 liter of sediment. For instance, if an 800 cc sample yields 2000 worms, this means 2500 in 1 liter.

Supplement to Memorandum No. 8

Points to be Covered in a Biological Survey of the Ohio River

1. Preliminary to the work, the map of the drainage area should be thoroughly studied. This will give an idea of the:

   Rapidity of run-off, which materially affects water age, hence biological productivity.

   Relative arable land conditions, materially affecting silt content of the water.

   Population density.

   General centers of industrial waste and their nature, e.g., mining areas, etc.

2. A determination should be made of the possible examinations. Since this cannot be determined by preliminary work, the following recommendations are made:

   A. Regular examination of plankton samples taken by collectors, and examined after centrifuging. Only those plankters should be counted which have been shown to be favorably or unfavorably affected by domestic sewage.

   It is recommended that 100 ml. of raw river water be taken from each composite sample, centrifuged 3 to 5 minutes at speeds between 2000 and 3000 r.p.m., the supernatant water decanted, and the catch measured, either as so many ml., or as so many drops, 1 ml. or 1 drop of catch having a definite ratio to the 100 ml. sample. If large plankters, as some of the larger Rotifera, are present in abundance, it is recommended that the catch be examined in a counting chamber, under a low-power binocular at 50 to 100 diameters. If such large organisms are few, it is recommended that an additional count be made by the drop method (previously published) and that the following species be counted:

Pollution-favoring

Endorina elegans
Chlamydobotrys gracilis
Pandorina morum
Euglena gracilis
" fusca

Euglena oxyuris
" viridis
Lepocinclis texta
Anthophysa vegetans
Collodictyon triciliatum

Carchesium spp.
Chilodonella uncinatus
Colpidium campylum
" colpoda
Colpoda cucullus

Colpoda aspera
Epistylis spp.
Glaucoma scintillans
" pyriformis
Lionotus fasciola

Paramecium caudatum
Stentor polymorphus
Vorticella spp.

Clean-water favoring

Chroomonas - all species
Chrysococcus - all species
Cryptomonas erosa
" ovata
Cryptoglena pigra

Dinobryon - all species
Domatomonas cylindrica
Euglena mutabilis
" sciotiensis
" spirogyra

Mallomonas caudata
Rhodomonas lacustris
Synura uvella
Actinobolus radians
Codonella cratera

Cyclidium spp.
Strobilidium humile
Strombidium spp.
Tintinnidium fluviatile
Attheya zachariaci

Melosira granulata
Melosira varians
Synedra biceps
Actinastrum hantschii
Ankistrodesmus falcotus

Other species occurring in large numbers should be counted if time allows, but may not be significant; Cyclotella, Chlamydomonas and possibly other species may occur in enormous numbers but not be significant. Hence, with lack of time, they can easily be preserved and possibly counted at another time.

B. The above plankton examination should reveal the sanitary condition of the water. But it should be supplemented by using a plankton net catch, which will include the crustacea and insect larvae which are not attached, also give a more accurate count of the large Rotifera, Nematoda, Gastrotricha, colonial protozoa and larger Algae and Desmids. This net catch should be made once weekly at the dams in the Ohio, but is not so important in the tributary streams. The catch should be preserved in 5% formalin, for eventual more careful examination.

C. Bottom dwelling forms. - See "Laboratory procedure with the mud samples."

D. It is recommended that traps holding glass slides suspended vertically be placed in the river at Cincinnati and at the Kiski to determine the rate of accumulation of attaching organisms. These can be examined at varying intervals.

E. Special examinations should be made of the plankton content, and also the bottom dwelling forms in tributaries to determine:

   a. Contributions to main river.

   b. Effects of specific trade wastes on those tributaries, as distillery wastes on Lawrenceburg Creek.

F. Isolated studies at various points during the course of the survey, to determine the immediate effect of industrial wastes, as a local effect. This would involve a survey above, one immediately below and one several miles (or hours flow) below the point at which the waste enters the stream.

3. A correlation of the important factors should be carefully sought as the survey progresses. For example, at times of low flow, the acid of the coal mining areas may travel far down the Ohio, but we as yet know nothing of its effects on life in the stream. Or a rain on the Licking may increase the turbidity of the Ohio greatly, without becoming an important diluting factor. But its effect on the plankton is to sharply decrease it.

Memorandum No. 9

June 7, 1939

Instructions Governing The Preparation Of Bacteriological Culture Media From Dehydrated Stock Powder

87-A

MEMORANDUM NO. 9

June 7, 1939.

INSTRUCTIONS GOVERNING THE PREPARATION OF BACTERIOLOGICAL CULTURE MEDIA FROM DEHYDRATED STOCK POWDER

It has been observed that difficulties have been encountered from time to time in the preparation of culture media from dehydrated products. These difficulties have been of two kinds - (1) Due to insufficient heating or to the formation of lumps when the dehydrated powder is added, the powder is not uniformly distributed when the mixture is prepared for sterilization. (2) Due to overheating or heating for too long a period, the finished media contains a precipitate which may be confusing. The contributing sources of error are extremely hard to detect when the media being prepared contains a dye.

Various types of preparatory methods have been tried out in detail and a procedure has been developed which (1) can be carried out rapidly, (2) avoids the difficulties referred to, and (3) produces a satisfactory, clear medium.

The instructions, which follow, are for a one liter portion of Standard nutrient agar. If larger amounts are to be prepared, the same instructions will apply multiplying the amounts given by the number of liters desired. The same instructions shall be applied to other varities of dehydrated media altering the amount of dehydrated powder employed per liter in accordance with the instructions given on the container.

Procedure for dissolving and preparing dehydrated Standard nutrient agar:

(1) Put 800 ml. of distilled water in pot or flask and apply heat.

(2) Weigh out 23.0 grams of dehydrated Standard nutrient agar, place in a beaker casserole or other suitable container and add 50. ml. of cold distilled water slowly. Mix well to a heavy paste, making sure that all of the powder is evenly wetted and free from lumps. When the water in (1) is boiling briskly, add an additional 150 ml. of cold distilled water to this paste and mix to give a free-flowing mixture.

(3) Add this concentrated agar suspension, (2), to the boiling distilled water, (1), stirring briskly and keeping the mixture boiling over a free flame. If the mixture has been prepared properly and is free from lumps, adequate solution of the agar will be obtained with 2 minutes (not to exceed 5) of boiling.

(4) Make up any loss in volume caused by evaporation.

(5) Distribute in containers in desired quantities and sterilize by autoclaving.

In the preparation with the apparatus available for use it may be found that the amount of cold water required for successfully carrying out step (2) may be decreased and the amount of boiling water in step (1) proportionately increased. If so this may be done for the smaller the volume of water used in (2) and the larger the amount in (1), the less the interference with the boiling temperature and the desired result will be obtained more quickly; providing always that a sufficien amount of cold water is used in (2) to obtain an evenly wetted, lump-free, free-flowing mixture.

Memorandum No.11

March 27, 1940

Information For Trailer Laboratory Crew

Memorandum No. 11

March 27, 1940

INFORMATION FOR TRAILER LABORATORY CREW

- General

Operation of the mobile laboratory units has indicated that umerous problems and difficulties may arise not ordinarily enountered in the case of fixed laboratories with more ideal orking conditions. This brief memorandum has been prepared ith the idea of guiding the personnel of the units with respect o their duties and responsibilities.

On the basis of the work done in 1939 and in view of the ecrease in number of tests to be made per sample in 1940, an verage daily examination of eight (8) samples, 5 days per week, s contemplated. The length of the working day will necessarily ary in this type of field work. In another section of this emorandum the principal duties of each member of the party have een listed in the order of their importance and priority. By roper use of time and close cooperation by members of the unit, t is believed that the daily laboratory routine can be carried ut in an eight hour day from Monday to Friday, 4 hours on aturday, and a few hours, as necessary, on Sunday to examine amples under incubation. Modification of the sampling schedule ay be necessary if the work cannot be completed in a 48 hour eek. Occasionally it may be necessary for one or all members f the party to work longer hours than indicated above. If at ll possible, without hindering the work, an equal time off hall be allowed for the extra time worked. The time off shall e taken within two weeks of the date the extra duty was perormed and shall not be cumulative so as to add to the annual eave of the individual.

Change of location of trailer headquarters should be voided on Sundays. However, Saturday moves should be made if ampling in a given area is completed by Friday of any week. aturday is to be considered as a full working day of eight ours, according to the regulations of the U. S. Public Health ervice.

The principal duties of the various members of the party e outlined below for the guidance of each member of the ailer personnel.

II - Duties of Personnel

Junior Chemist:

1) Supervise and direct the work of sample collector and laboratory attendance.

2) Program the work with the guidance and assistance of the engineers from the Stream Sanitation Office and the Stream Pollution Investigations Station.

3) Supervise and help in preparations for moving.

4) Supervise and help in making set-ups on new locations, making all contacts with local authorities personally, if at all possible.

5) Arrange with local authorities for use of any of their equipment or facilities - water, power, distilled water, etc.

6) Carry on analytical work with such assistance as laboratory attendant can give when he has carried out his primary duties as outlined below.

7) Complete laboratory records on analytical results, with help of laboratory attendant, within one week of completion of work at a given set-up, and send cards and general report covering the work to Cincinnati headquarters.

8) Inform the Cincinnati office of all matters connected with payroll information and per diem - annual or sick leave of any member of party - time of leaving and arriving at various places with trailer, etc.

9) Send in on the last day of each month a tabulation showing: -

    A - Number of samples collected - (each regular collection at a sample point represents 4 samples - one is the bacteriological sample, one is the composite sample, and each D.O. bottle is a sample; four in all.

    B - Number of Bacteriological examinations for B. coli.

C - Number of Bacteriological examinations for total count.

D - Number of D. O. analyses made.

E - Number of B. O. D. Analyses made.

F - Number of Turbidity Determinations Made.

G - Number of pH " "

H - Number of Alkalinity " "

I - Number of Hardness " "

J - Number of Fe tests made

K - Number of Acidity Determinations made.

L - Number of Nitrite " "

M - Number of Any Special Determinations made.

Chauffeur:

1) Drive tow car and trailer unit from place to place and connect and disconnect tow car, etc., with help of other members of the party.

2) Sample collections.

3) Care of car and trailer, washing, grease & servicing, etc.

4) Help in making set-ups on new locations.

5) Call at post office for mail and express.

6) Ship cases, etc.

7) Help in dishwashing and laboratory work as instructed by Jr. Chemist to complete working day after all the above duties have been carried out.

Laboratory Attendant:

1) Prepare and sterilize media.
2) Wash and sterilize glassware.
3) Prepare laboratory reagents.
4) Help preparing for moving trailer and in making set-ups on new locations.
5) Assist Jr. Chemist in analytical work, as directed, when above duties have been completed.
6) Assist Jr. Chemist in completing records on the laboratory cards.

III - Proposed Schedule of Laboratory Work

The routine tests contemplated are:-

1) Temperature.
2) Dissolved Oxygen - Azide Method.
3) 5-Day B. O. D.
4) Coliform Index - Dilution Method.

The following determinations shall be made occasionally as explained below: -

1) Alkalinity.
2) Turbidity.
3) Hardness (Soap Method).
4) Iron - (Total, Ferrous, Ferric).
5) Acidity to phenolphthalien (hot and cold).
6) pH.

Alkalinity shall be run on at least one sample from each sampling station. Turbidity and hardness shall be run on all samples at one station on each stream (preferably lowest point downstream). Iron, acidity and pH tests shall be made only on stream samples where acid mine drainage or other acid discharges result in pH values below 4.0, or where iron bearing wastes are present in appreciable amounts.

Methods to be followed in making these determinations shall be in accordance with Memorandum No. 4 on "Outline of Chemical Methods" and any supplemental instructions on specific procedures issued from the Cincinnati station. Where there is any doubt as to tests not covered in these references, the procedures in "Standard Methods" shall be used.

IV - Detailed Instructions

The actual time of reporting to work and leaving, lunch hour, etc., will vary with the duties of the individual. The sample collector will start his trips so as to be back with samples by 1:00 P. M. If it appears desirable, the Jr. Chemist and laboratory attendant may stagger their shifts. This arrangement indicated certain advantages during the work carried on in 1939.

Tow car shall be garaged at the trailer location (usually a water plant) if not located too far from the living quarters of sample collector so as to be within reasonable walking distance. If there are no facilities available at the water plant for car storage, or if too far from town, the tow car shall be garaged at a public garage and voucher obtained by the chauffeur for this expense. The tow car shall be used only for official purposes. The government shall not provide transportation for any member of the party between his living quarters and the trailer location. When trailer headquarters are changed, the chauffeur and laboratory attendant will use the tow car for their transportation. The Jr. Chemist will have travel authority allowing use of his car or of government transportation requests to take care of his transportation.

It may be necessary and desirable at times for the Jr. Chemist to make field trips to sampling points to carry on dissolved oxygen tests,etc., in order to properly locate certain sampling points. Whenever possible these trips shall be made either with the assistant public health engineer assigned to the trailers or in the government tow car. If either of these two methods of transportation are not available he shall use his private car and report the mileage traveled and all necessary information on the regular voucher form provided for this purpose. A statement explaining the necessity for the trip and work done shall accompany this voucher in order that travel orders can be prepared covering the particular travel or trip involved.

Purchases may be made for any reasonable item, and emergency repairs can be taken care of in the field. Vouchers for such expenditures should be obtained. If possible, government purchase orders shall be used for such expenditures.

The success of the mobile laboratories depends upon cooperation of all members and a good morale. These instructions are offered as a mechanical basis from which to work, but the success of the program depends upon each of the party members themselves.

Memorandum No.12

May 6, 1940.

Supplemental Chemical Procedures For Upper Ohio River Samples

95-A

MEMORANDUM NO. 12

May 6, 1940.

SUPPLEMENTAL CHEMICAL PROCEDURES FOR UPPER OHIO RIVER SAMPLES

In the upper Ohio River and its tributaries acid iron bearing water will very likely be encountered. For this reason the following chemical tests and special procedures shall be followed to supplement and/or replace the procedures used in the middle Ohio River.

pH

The pH should be determined first either electrometrically or colorimetrically with an S.D.C. kit upon all samples. If the pH is 6.0 or above, total iron and one B.O.D. determination upon the water as received without pH adjustment or seeding should be made. But, if the pH of the sample is below 6.0, both total and ferrous iron and two B.O.D. determinations should be made. These B.O.D. determinations should be made as follows:

I. Upon one portion of the sample as received without pH adjustment or seeding.

II. Upon another portion following pH adjustment to 7.2 with N/10 Sodium Hydroxide and seeding with about 2.0% of normal river water (pH 6.8 to 8.0). The details of this procedure follow later.

Acidity

If the pH is below 6.0, the acidity to phenolpthalein of both hot and cold samples should be determined. When the pH is below 5.1 the phenolpthalein acidities and the acidity to methyl red should be determined. (See Standard Methods)

Iron

Total iron may be determined by boiling 50 ml. of sample with 5 ml. of 6 N nitric acid for 5 minutes, adding 3 drops of permanganate solution and cooling. Add 5 ml. of thiocyanate and compare immediately with standards prepared from standard iron solution with 6 N nitric acid. (Standard Methods p. 75) If the sample gives evidence of considerable pollution (is septic) the evaporation and ignition procedure of Standard Methods should be resorted to. (item 2.1 p. 74)

Ferrous Iron

Ferrous iron should be determined when the pH of the sample is less than 6.0 by the procedure given on page 76 of Standard Methods. As one part of ferrous iron results in an oxygen loss of 0.14 ppm in a neutral water and ferric iron increases the apparent oxygen content during the final acidification by the Winkler procedure, it is necessary to take the proper precautionary measures in determining dissolved oxygen in iron bearing waters.

Dissolved Oxygen

Because of the possibility of iron in the upper Ohio River and tributary water and the interference noted above, the Rideal Stewart procedure employing the introduction of 2 ml. of 40 per cent of KF. $2H_2O$ following the sulfuric acid and previous to the permanganate solution should be used for all dissolved oxygen determinations from new sources on the upper Ohio River system. If later experience upon water from any location consistently shows that samples have a pH above 6.0, are not septic and contain less than 10 ppm of total iron (under these conditions ferrous iron should not be present) the sodium azide modification for D.O. may be employed. However, it should be noted that the production of a faint straw-yellow color during the preliminary treatment with acid azide indicates the presence of considerable ferric iron. About 40 ppm of ferric iron can be detected by a faint color in a D.O. bottle. Larger quantities of iron increase the color intensity. Consequently the appearance of a straw or brown color during acid azide treatment indicates that the method is not applicable and that the use of potassium fluoride with the Rideal Stewart method the following important details of procedure must be carefully followed if dependable dissolved oxygen data are to be obtained. Follow the procedure outlined in Standard Methods item 2.1 page 147. Add 2 ml. of potassium fluoride and continue exactly through item 2.4. After the addition of the potassium oxalate the samples must be allowed to decolorize in the dark. The procedure should not be completed as soon as decolorization is apparently complete, but the samples should be allowed to stand for an additional 30 minutes in the dark before the manganous sulphate and alkaline potassium iodide are added. After shaking and settling twice, samples are ready to be acidified and titrated. If for any reason the samples cannot be titrated immediately, they should be allowed to stand in the alkaline state until they can be titrated. Then they should be acidified, one at a time, using 2 ml. of concentrated sulphuric acid in place of the phosphoric acid suggested in Standard Methods, and titrated immediately.

B.O.D. Procedure II

The following B.O.D. procedure is to be applied as a supplementary test to the regular procedure where the pH of the sample is below 6.0. While a B.O.D. will be obtained in the pH range below 6.0, it is not due to the normal microflora and fauna. It is therefore necessary to make a pH adjustment to the normal biochemical range and reseed to obtain the normal B.O.D. A portion of the sample should be carefully adjusted with N/10 - NaOH until the pH is between 7.0 and 7.8. As this neutralization proceeds a heavy precipitate of ferric hydrozide may be obtained where much iron is present. When this occurs it is best to allow the precipitate to settle and check the pH on the supernatant. An attempt should be made to bring the pH to 7.2 but as long as pH 7.8 is not exceeded it is not necessary to readjust the pH with acid. After the pH has been adjusted, the sample should be seeded with 2.0% normal river water. If normal river water is not available, one ml. of domestic sewage filtered through paper (Whatman number 1) should be added per liter of sample. In either case the same concentration of seed used on the sample shall be put into dilution water and its B.O.D. determined so that a correction for seed can be made in the final B.O.D. calculations. In all cases where seeding is necessary, the B.O.D. obtained on the seed should be reported. The sample for B.O.D. should not be diluted until experience at the sampling point indicates that the dissolved oxygen will be depleted without dilution. In all cases where this supplemental B.O.D. after pH adjustment is made, the Rideal Stewart procedure employing potassium fluoride as described shall be used for all D. O. determinations.

APPENDIX II

SPECIFICATIONS FOR MOBILE LABORATORY UNIT

APPENDIX II.

SPECIFICATIONS FOR MOBILE LABORATORY UNIT

1. SERVICE REQUIREMENTS

The trailer specified herein is for use by the United States Public Health Service as a mobile laboratory unit. It will be moved from place to place by the tractor unit which will be furnished by the Government and delivered f.o.b. the railroad station nearest the contractor's delivery point. The tractor unit shall be unloaded and serviced by the contractor, who shall make any alterations necessary for installation of towing irons on the tractor unit. The cost of unloading, servicing and alterations, if any, shall be included in the bid price. Installation of helper springs on the tractor unit shall be done by the trailer contractor.

The trailer and its tractor unit will travel to various parts of the United States over all kinds of roads and under various weather conditions which will necessitate unusually sturdy construction. Provision shall be made for adequate lighting, ventilation, plumbing, etc. as set forth in these specifications. Both plans and specifications form an integral part of the contract. Details described under specifications are equally binding as if shown on the plans and vice versa.

2. GENERAL DESCRIPTION

The unit shall be of the trailer type with a single rear axle equipped with single tires. The trailer shall be fully enclosed and provided with one side door near the front of the right side wall. Windows and door shall be installed as explained elsewhere in these specifications.

The trailer shall have the following minimum dimensions:

Inside length exclusive of nose section shall be not less than 15' 0".

Inside width exclusive of nose section shall be not less than 74".

Inside height exclusive of nose section shall be not less than 78"

Outside length overall, not less than 17' 2"

Outside width " " " " 78"

Outside height " " " " 99"

Bidders must state the exact dimensions of the trailer they propose to furnish.

The center line of the axle shall be so located as to provide a load space length along the floor of not less than 7 ft. 6" between the front edge of the front wheel housing and the front of the trailer at its widest width. Vertical load on draw bar for the trailer shell shall not exceed 300 lbs.

3. FRAME AND BRACING

The trailer shall be equipped with an all steel chassis running the entire length of the under structure, with secure anchorage at points of spring attachment and sidewall attachment. The sidewall structure shall be securely attached and braced to the underbody so that the outside sheeting material when applied will form a rigid shell. All sidewall structural members shall be securely fastened together by cadmium plated bolts or screws, to prevent rusting.

4. FLOOR, WALLS AND ROOF

The floor shall be made of double 5 ply floor paneling having a total thickness of one inch, and shall be covered by a good grade of linoleum at least 1/8 inch in thickness.

The outside walls from the lower rub rail to the roof rail shall be sheathed with 3/16" ply metal. This material shall consist of a sheet of rust proofed steel glued with a suitable water proofed bond to plywood built up in similar fashion.

The curved roof bows shall be made of thoroughly seasoned spruce or equal placed close enough together and braced longitudinally so as to form a rigid roof construction. The roof bows shall be covered with 3-ply fir or equal plywood not less than 3/16" in thickness, painted with an asphaltic paint and then covered with No. 10 extra heavy waterproof canvas, treated with aluminum paint, suitable for outside exposure.

The interior side walls and ceiling shall be covered with 3-ply fir or equal plywood not less than 3/16" in thickness and of kiln dried, low moisture content, stock. Space between inside and outside linings of roof and side walls shall be insulated against heat by a combination of not less than 1-1/2" of dead air space filled with approved insulation such as spun glass or equal. Floor and wheel housings shall be insulated with celotex and padding.

5. AXLES, WHEELS, TIRES

The axle shall be of a suitable alloy steel forged in one piece and equipped with the necessary hubs for the installation of wheel equipment. The axle capacity shall be ample to carry the load of trailer shell and a payload of 2,000 lbs. The unit shall be equipped with suitable, 12 leaf springs constructed of a high grade of silicon manganese steel. The wheels shall be constructed of pressed steel of the closed disk type, with chrome-plated hubs, and the outward appearance of same shall be in close conformity with the exterior appearance of the trailer unit. The tires furnished shall be of the proper size to accommodate the total weight of the trailer and equipment (payload 2,000 lbs.) and shall be rated in accordance with the latest tire and rim association standards.

Spare tire, tube, and rim or wheel shall be provided and suitably mounted at the rear of the trailer unit. The tire carrier shall be equipped with a suitable locking device, complete with two keys. Tools for making a tire change shall be furnished.

6. WINDOWS, DOORS, AND VENTILATORS

The trailer shall have eight windows located as shown on the plans, and of the sizes indicated there. The windows shall be of the drop type and all glass in the trailer shall be shatterproof glass. The trailer door shall have a window as shown on plans. A folding screen door shall be installed at the trailer doorway. Screen door, all drop windows, and ventilator opening shall be provided with removable 16 mesh copper screens. Each window shall be provided with a good grade roll shade of color to harmonize with the trailer color.

Roof ventilator opening shall be located as shown on plans with hinged hatch that may be opened to desired height from inside the trailer. An electric ventilating fan, with capacity of 200 cu. ft. of air per minute shall be provided and installed preferably in the roof ventilator space. Roof ventilator shall be built above the outer roof level if necessary to accommodate the fan. The fan unit shall not extend into the trailer below the inside ceiling surface. The fan shall operate satisfactorily on either 110 volt, 60 cycle, single phase alternating current or on 110 volt direct current. A switch to control the fan operation shall be provided.

7. BRAKES

The trailer shall be equipped with Warner Electric Brakes, or equal, having heavy duty brake linings, and with a Warner Brake Control Kit, or equal. The brakes shall be installed with all accessories and attachments, complete and ready for use. An adequate safety brake shall be furnished for locking the trailer wheels in case of emergency and for parking.

8. ELECTRICAL

(a) Lights - 6 volt system.

The trailer shall be equipped with outside running lights to conform with Interstate Commerce Regulations and in addition equipped with combination "turn" signal and stop and tail lights. All of these lights and a central dome light in the trailer shall be connected to the tractor car through a convenient connector and operate with the tractor car light switch.

(b) Wiring and General (110 volt electrical current; may be either direct or alternating).

All wiring electrical work and fixtures shall meet the requirements of the National Electrical Code.

Opening on the outside of the trailers, flush with the finished surface, there shall be installed a weather and dust proof electrical receptacle through which the trailer shall be supplied with electrical current from an outside source. The 110 volt electrical current shall be supplied to the trailer from an outside source by plugging in the 150 foot electrical cable specified elsewhere. The outside receptacle shall be wired to a "Nofuze Load Center" located in the interior of the trailer at a convenient and accessible point. It shall also be located within one or two feet of the outside receptacle.

The load center shall be of the type made by either Graybar Electric Company or Westinghouse Company; the most compact unit available shall be selected. This load center shall have four single pole circuits. Three shall be of 15 amperes capacity each, and the fourth of 20 ampere capacity, The 15 ampere circuits shall be wired to the following units:

(1) Seven 110 volt ceiling and wall lights, and ventilating fan,

(2) The two double wall receptacles on one side of the trailer,

(3) The two double wall receptacles on opposite side of trailer.

The 20 ampere circuit shall be wired to a double base receptacle located at the base of the trailer near the door, as shown on the drawings.

Four double electrical wall receptacles shall be installed as shown on drawings.

Interior lighting shall be provided by seven light fixtures located as indicated on the drawings. These fixtures shall be firmly fastened to the trailer shell and shall be of a type with bulb setting in a horizontal position and having an adjustable cylindrical shade or reflector with bronze finish on the exterior and aluminum paint finish on the interior reflecting surface. Each fixture shall be an individual pull chain switch, and all seven shall be controlled by a single switch located at the side of the trailer door.

The contractor shall furnish 150 ft. of stranded electrical cord insulated with heavy live rubber, weather proofed, with plugs and attachments which shall carry the current from an outside source to the receptacle at the trailer. This cord or cable shall be of ample capacity to supply safely the current required to operate the present and future load which will be a total of 4500 watts.

The attachment plug on one end of the cable shall fit into the receptacle provided in the front end of the trailer. The other end of the cable shall be fitted with a substantial two-pronged plug (with parallel prongs), for tapping into a standard 110 volt wall receptacle.

The dome light, shown on drawing, (with independent switch at side of door) tail lights and side clearance lights shall operate on 6 volt direct current from tractor car power system. Wiring and connections shall be installed independently of the 110 volt system. An outside receptacle shall be installed at the front end of the trailer for connection of the 6 volt trailer circuit to the tow car circuit. A connecting cable shall be supplied for use with a standard Ford, Chevrolet or Plymouth coupe as tractor car, and the trailer system shall be controlled by the tractor car light switch.

9. WATER SUPPLY

At the rear end of the trailer under rear sink, a 30 gallon water storage tank shall be installed as indicated on the plans. The tank shall be made of rust resistant materials and shall have two baffle plates built into it to minimize water motion and swaying. The tank shall have an outside spout for filling, and a drain plug in the bottom for draining it, accessible from the outside.

In addition to the tank water supply, provision shall be made at the rear of the trailer for a 3/4" hose connection to city pressure. On the outside this hose receptacle shall be equipped with a chrome plated cap attached to the trailer with a chain. From the inside, a copper tubing connection shall be made to a goose neck spigot. This fixture shall be installed at the sink at rear of the trailer. The piping, gooseneck spigot and hose receptacle shall be provided and installed by the trailer contractor. This system shall be designed to operate without leaks or trouble at a pressure of 150 lbs. per sq. inch.

All copper tubing used for water supply lines to faucets and tanks shall be housed and protected by wooden framing wherever they pass through cupboards or lockers.

10. INTERIOR FURNITURE AND EQUIPMENT

(a) Front and rear lockers.

Rear cove locker and front end closet and lockers shall be built in as indicated on the plans. These units shall be made of plywood and shelves shall be installed as indicated.

A 5-1/2" shelf with cleat 1/4" thick, and rising 1/2" above top of shelf level on ends and front edge, shall be installed as shown on plans.

(b) Laboratory Benches.

The laboratory benches indicated on plans shall be built into the trailer. Drawers, cupboards, and open spaces beneath bench tops shall be provided as shown on plans. All drawers and cupboard doors shall have adequate fasteners installed so they will remain shut during movement of trailer without the necessity of locking them. This furniture may be constructed of plywood of suitable thickness except the bench tops. These shall be made of one inch clear douglas fir or equal, and shall have dowelled and glued joints so as to prevent opening of cracks. The bench tops shall be left unpainted but doors, drawers, etc. shall be stained a light oak color, and varnished to match interior wall finish of trailer. (Bench tops will be treated with acid proof paint by the U. S. Public Health Service after delivery of trailers.) There shall be at least 36" clearance from under surface of bench tops to the floor. If possible, so as not to extend benches above window sill, this clearance shall be 38".

The doors of the cupboard over the wheel housing shall have a clear opening 8" x 10" in size. This opening shall be covered by a 4 mesh brass screen neatly fastened to the doors from the inside. An opening 5" x 10" in size shall be provided in the wheel housing in this cupboard as shown on the drawings. This opening shall be screened with an 8 mesh brass screen. A hinged cover with a latch shall be placed over this opening inside the cupboard. The opening is to be used for providing ventilation to the 20° incubator compressor unit which is to be installed in this cupboard.

(c) Ice Box Unit.

Over the wheel housing opposite the door, an ice box and sink unit of standard trailer design shall be installed - plans indicate exact location. This unit shall have a 12" x 18" porcelain sink with drain and stop. A double action water pump shall be installed at this sink with copper suction line to water storage tank at rear of trailer.

The ice box shall be provided with an ice compartment having a capacity of not less than 40 lbs. of ice. The food compartment shall have one shelf at mid-height of the storage space. An outside drain from the ice compartment shall be provided. Ice and food compartment shall be made of rust resistant materials.

(d) Rear Bench, Sink and Covering.

In the bench at the rear of the trailer a sink and lead covering on bench shall be installed. The sink shall be located as indicated on the plans and shall be similar and equal to that made by Kewaunee Manufacturing Company, Adrian, Michigan, and shown on p. 35 of their 1935 catalog, as sink No. S-755. This is a Karcite sink with dimensions as follow:

	Length	Width	Depth
Inside	22"	14"	6"
Outside	24½"	16½"	7-1/8"

The top of the sink cabinet and adjoining bench, as indicated on plans, shall be covered with sheet lead consisting of Tellurium lead weighing not less than 4 lbs. to the square foot and shall be sloped to drain into the sink. All joints shall be burned with pure lead. This lead sheet shall extend over the curb and into the sink opening. A raised bead shall be formed 1/4" high around all four edges of the sink bench and drainboard, to prevent spilled acids from dripping onto the floor. This bench top shall have a drip groove in the under side of the top at the outer edges and around sink opening to prevent capillary attraction carrying water into the case work. No solder shall be used in making any of the joints. Sink drain shall be of extra heavy tellurium lead, and all joints shall be burned with pure lead. No solder shall be used. The sink drain shall discharge to the ground and be convenient of access for connection of a hose when necessary or desirable to lead sink drainage to a sewer. A double action suction pump shall be installed at the rear sink, as shown on plans, with connection to the underside of water tank for delivering water to the sink. As noted under "Water Supply," this sink shall also have a goose neck spigot fixture to be supplied by city water pressure.

11. MISCELLANEOUS

Trailer contractor shall supply a two burner gasoline stove. This shall be a Coleman 2 burner stove #392, or equal.

The trailer contractor shall supply two one quart size Pyrene Fire extinguishers or equal. with necessary wall clamps.

A trailer jack shall be provided by trailer contractor with a caster wheel, raising and lowering on a worm thread acting as a jack, to raise or lower front end of the trailer.

A pair of heavy duty passenger car type tire chains of the proper size for the trailer tires shall be furnished by the trailer contractor.

The trailer contractor shall furnish four stabilizing jacks of ample strength to steady the trailer unit against movement when it is set up for laboratory use. The following jacks or equal will be acceptable:- "E-3 Jack Twins" manufactured by Wiedman Specialty Company, 20 Clinton Street, Tonawanda, New York.

Drawbar, Coupler, and Tow Iron

Trailer contractor shall install draw bar equipment, firmly anchored to trailer underbody, as part of trailer contract. This draw bar shall be fitted with a ball and socket type of coupler, which shall be of ample strength to transfer trailer load to tractor unit. Safety chain shall also be supplied to augment this draw-bar coupling.

A vent opening with vent pipe, flashing and hood, installed, shall be provided. The vent shall not extend into the interior of the trailer but shall be capped temporarily. This vent unit is to be provided for future use as a stove vent. It shall be installed in the position indicated on the plans.

12. TRACTOR UNIT

The tractor unit shall be a new coupe automobile furnished by the Government and delivered to the trailer contractor's plant. The trailer contractor shall make any necessary alterations to accommodate the trailer coupling device, the cost of alterations to be included in his bid price. The trailer car contractor shall make all necessary electrical, brake and draw bar connections. The tractor unit shall be equipped by the trailer car contractor with the necessary iron bar by which the trailer shall be drawn. This bar shall be attached rigidly to at least two cross frame members of the tow car frame. It shall be of ample strength to support safely a draw bar load of 600 lbs. Helper springs shall be installed on the tractor car by the trailer contractor, and shall adequately reinforce the rear springs of the tractor car for the load to be carried.

It shall be permissible for the trailer contractor to drive the tractor car under its own power provided:-

(1) The helper springs, iron tow bar, etc. cannot be installed at the contractor's plant, and

(2) That the total distance traveled for having such installations made shall not exceed 50 miles.

13. UNITS TO BE SUPPLIED BY CONTRACTOR

Trailer contractor shall furnish trailer built to conform to these specifications and shall include all furniture such as cabinets, cupboards, drawers, ice box and sink unit, capped vent opening for stove, water tank and necessary piping for both sink faucets, electrical wiring and receptacles, nofuze load center, 150' of electrical connecting cable, a 2 burner gasoline stove, 2 one-quart fire extinguishers, ventilating fan, tire chains and trailer jacks, all as specified.

14. UNITS TO BE SUPPLIED BY U. S. PUBLIC HEALTH SERVICE

(To be installed by U. S. Public Health Service after delivery of trailer)

1 autoclave,
1 20° C. Incubator,
1 37° C. Incubator,
1 Hot Air Sterilizer,
1 Electric Hot Plate,

15. COLOR AND LETTERING

The exterior finish shall be high-grade, automotive gray enamel. Exterior shall be painted gray and shall receive one primer coat, two color coats and one finishing coat. Within one week of award of the contract the successful bidder shall provide a sample color plate of the finished trailer color to the Government in order that it may be forwarded to the tractor car contractor for the purpose of matching the colors of the two units.

The words:-

Federal Security Agency
U. S. Public Health Service
Official

shall be placed in gold leaf letters with black outlines on each side of the trailer. They shall be placed on the door of the trailer on one side and directly opposite on the other. The words "Federal Security Agency" shall be in letters 7/8" high; the words "U. S. Public Health Service" shall be in letters 1¼" high, and the word "Official" shall be in letters 5/8" high. All lettering shall be painted over with two coats of durable outside spar varnish.

16. INSPECTION AND DELIVERY

After all the connections have been made to the tractor unit, a representative of the Government will inspect the trailers, tractor cars and connections. At that time, if everything is satisfactory, the entire train shall be turned over to this inspector or shipped on Government Bill of Lading to the United States Public Health Service, Cincinnati, Ohio.

Due to the urgency of obtaining these units the bidder shall state the time in which delivery can be made. This delivery time will be duly considered in making the award of contract.

APPENDIX III

LABORATORY FORMS

LABORATORY FORMS

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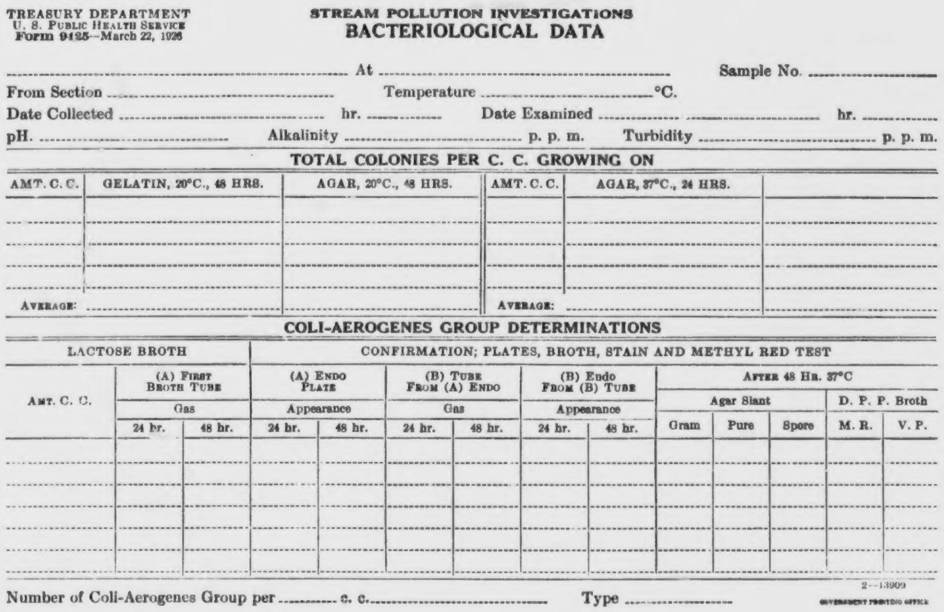

TREASURY DEPARTMENT
U. S. PUBLIC HEALTH SERVICE
Form 9425—March 22, 1926

STREAM POLLUTION INVESTIGATIONS

BACTERIOLOGICAL DATA

.... At .... Sample No. ....

From Section .... Temperature ....°C.

Date Collected .... hr. .... Date Examined .... hr. ....

pH. .... Alkalinity .... p. p. m. Turbidity .... p. p. m.

TOTAL COLONIES PER C. C. GROWING ON

AMT. C. C.	GELATIN, 20°C., 48 HRS.	AGAR, 20°C., 48 HRS.	AMT. C. C.	AGAR, 37°C., 24 HRS.	
AVERAGE:			AVERAGE:		

COLI-AEROGENES GROUP DETERMINATIONS

LACTOSE BROTH			CONFIRMATION; PLATES, BROTH, STAIN AND METHYL RED TEST										
AMT. C. C.	(A) FIRST BROTH TUBE		(A) ENDO PLATE		(B) TUBE FROM (A) ENDO		(B) Endo FROM (B) TUBE		AFTER 48 HR. 37°C				
									Agar Slant			D. P. P. Broth	
	Gas		Appearance		Gas		Appearance						
	24 hr.	48 hr.	24 hr.	48 hr.	24 hr.	48 hr.	24 hr.	48 hr.	Gram	Pure	Spore	M. R.	V. P.

Number of Coli-Aerogenes Group per .... c. c. .... Type ....

2—13909
GOVERNMENT PRINTING OFFICE

Form No. 1

Form No. 2

UNITED STATES PUBLIC HEALTH SERVICE

OXYGEN DEMAND AT 20° C.

Source of Sample ....

.... *Date* ...., *19*......

SAMPLE	DAYS INCUBATED	PER CENT CONCENTRATION	BURETTE READING C. C.		DISSOLVED OXYGEN P. P. M.				OXYGEN DEMAND
			INITIAL	FINAL	INITIAL	FINAL	DEPLETION		
							ACTUAL	COR.	

Form 9437—Federal Security Agency—Public Health Service—Oct. 1939 GPO 16—12871

Form No. 3

U. S. PUBLIC HEALTH SERVICE
OHIO RIVER POLLUTION SURVEY

Chemical Data

Laboratory ____________ Date ________________ 19__

SAMPLE NO.	SOURCE OR STATION NO.	pH	Turbidity	ALKALINITY DET. ON 100 ml. PORTION		DISSOLVED OXYGEN	
				Burette Reading	Alka p.p.m.	Method Used	Burette Readings ml.

U. S. Public Health Service - Ohio River Pollution Survey

ACID STREAM WATER OXYGEN DEMAND AT 20° C.

Source of Sample ______________________________

Sample No. ______________________ Date ______________________

Sample Incubated as Collected

Ferrous Iron	Initial	Final	Loss During Incubation
Sample ml.			
Standard Matched			
Titre - P.P.M.			

B. O. D. (D. O. method used ________________)

Days Incubated	% Concentration	Burette Reading ml.		Dissolved Oxygen P.P.M.				Oxygen Demand
						Depletion		
		Initial	Final	Initial	Final	Actual	Cor.	

Sample Incubated after pH adjustment and innoculation

Initial pH ____ pH after adjustment ____ Seed used ________

Days Incubated	% Concentration	Burette Reading ml.		Dissolved Oxygen P.P.M.				Oxygen Demand
						Depletion		
		Initial	Final	Initial	Final	Actual	Cor.	

Form No. 5.

U. S. Public Health Service - Ohio River Pollution Survey

ACID STREAM WATER CHEMICAL DATA

Laboratory________________ Date________________

Source________________ Sample No.________________

pH__________ Turbidity__________ Color__________

ALKALINITY			HARDNESS		
Sample ml.	Bur.Reading	P.P.M.	Samp. ml.	Bur. Read.	P.P.M.

ACIDITY

Method	Sample ml.	Burette Readings	P.P.M.
Methyl Red			
Phenolphthalein Hot			
Phenolphthalein Cold			

TOTAL IRON

Sample Ml.	Standard Matched or Burette Readings	P.P.M. Iron

Form No. 6.

U. S. Public Health Service - Ohio River Pollution Survey

ACID MINE WATER CHEMICAL DATA

Sample____________________________ Source____________________

pH_______________________ Sulphate__________________p.p.m.

ALKALINITY

Method	Sample-ml	Readings	Alkalinity p.p.m. as $CaCO_3$
Methyl Red			
Methyl Orange			

ACIDITY

Method	Sample ml	Burette Readings	Acidity p.p.m.as $CaCO_3$	Electrometric pH at "E. P."
Methyl Red				
Phth. Hot				
Phth. Cold				

TOTAL IRON

Sample-ml	Standard Matched or Burette Reading	p.p.m. Iron

(1) R_2O_3____________________p.p.m. (2) R_2O_3- 1.43 Fe___p.p.m.

(3) (A-1) 0.529 (2)_______________p.p.m.

Form No. 7.

U. S. Public Health Service - Ohio River Pollution Survey

ANALYSIS OF RIVER MUDS

Sample # ______________ From ______________________________

Date Sampled ____________________ Date Received ____________________

Condition ____________________ Odor ____________________

Appearance ____

Det. of Moisture and Volatile Solids

(1) Tare + Sample ____________ Moisture $\frac{(1) - (2) \times 100}{(1) - (4)}$ = ____________%

(2) Tare + Dry Mud ____________

(3) Tare + Ash ____________ Vol. Matter $\frac{(2) - (3) \times 106}{(2) - (4)}$ = ________ ppm.

(4) Tare ____________

Oxygen Consumed Data

(8) Sample ____________ grams "Apparent Immediate Demand"

(9) C.C.$K_2Cr_2O_7$ ______ (10)C.C.$FeSO_4$ ______ Dilution ____________________

$\frac{(9) - (10)}{(8)\ (M/100)} \times F_2$ = ____________ ppm D.O.Differential (D) ______________

"A.I.D."= $\frac{D \times \text{Dilution Factor}}{(M/100)}$

Organic Nitrogen

(11) Sample ________________ grams

(12) __________ C.C. __________ N Acid (13) __________ C.C. __________ N alkali

Nitrogen - $\frac{(12) - (13)}{(11)\ M/100} \times F_3\ F_4$ = ______________% Nitrogen

B. O. D. - Data

Days	B. O. D. ppm (Dry)
1	__________
3	__________
5	__________
7	__________
9	__________
___	__________

Grit ______ % ____________________________

____

Remarks ____________________________________

____

____

____

____

____

Form No. 8

U. S. Public Health Service - Ohio River Pollution Survey

BIOLOGICAL EXAMINATION

Watershed ______________________ Mileage ____________________

Subwatershed ______________________ Mileage ____________________

Sheet 1 of 4

Station	1	2	3	4	5	6	7	8	9	10	11	12
Location												
Mileage												
Date												
Agar Count												
B.O.D.												
D.O.												
pH												
Plankton (No. per cc.)												
Algae												
Chrysophyceae												
Chromulina sp.												
Chrysococcus rufescens												
" major												
" ovalis												
" asper												
Mallomonas caudata												
" sp.												
Hymenomonas roseola												
Synura uvella												
Dinobryon sertularia												
Lagynion Scherffelii												
Total												
Cryptophyceae												
Rhodomonas lacustris												
Chroomonas spp.												
Cryptomonas erosa												
" ovata												
Total												
Euglenophyceae												
Euglena viridis												
" pisciformis												
" sanguinea												
" variabilis												
" Ehrenbergii												
" acus												
" oxyuris												
" torta												
" tripteris												
" deses												
" polymorpha												
" gracilis												

Sheet 2 of 4

Station	1	2	3	4	5	6	7	8	8	10	11	12
Lepocinclis ovum												
" texta												
Phacus longicauda												
" anacoelus												
" pleuronectes												
" triquerter												
" pyrum												
Trachelomonas volvocina												
" crebea												
" hispida												
" urceolata												
Cryptoglena pigra												
Peranema sp.												
Petalomonas mediocanellata												
" carinata												
Total												
Chlorophyceae												
Mesostigma viride												
" grande												
Heteromastix angulata												
Chlamydomonas spp.												
Chlorogonium sp.												
Sphaerellopsis fluviatilis												
Thorakomonas sp.												
Wislouchiella planctonica												
Labomonas rostrata												
Coccomonas orbicularis												
Phacotus lenticularis												
Pteromonas aculeata												
" cruciata												
Polytoma uvella												
Gonium pectorale												
" sociale												
Pandorina morum												
Eudorina elegans												
Total												

Sheet 3 of 4

Station	1	2	3	4	5	6	7	8	9	10	11	12
Diatomeae												
Synedra acus												
" radians												
" biceps												
" ulna												
Cyclotella spp.												
Navicula spp.												
Asterionella formosa												
Melosira												
Gomphonema												
Nitzschii												
Total												
Miscellaneous												
Actinoastrum												
Anabaena												
Ankistrodesmus sp.												
Botryococcus												
Chroococcus												
Closterium												
Coelastrum												
Cosmarium												
Crucigenia												
Dictyosphaerium												
Golenkinia												
Merismopedia												
Microcystis												
Oocystis												
Oscillatoria												
Pediastrum												
Kirchreriella												
Scendesmus sp.												
Sorastrum												
Staurastrum sp.												
Total												
Protozoa												
Amoeba												
Codomonas annulata												
Oicomonas sp.												
Domatomonas cylindrica												
Bodo sp.												
Cladomonas sp.												
Vorticella sp.												
Podophrya												
Codonella cratera												
Prorodon sp.												
Colpodium Colpoda												
Urotrichia farcta												

Sheet 4 of 4

Station	1	2	3	4	5	6	7	8	9	10	11	12
Tintinnidium fluviatile												
Collodictyon triciliatum												
Glaucoma scintillans												
Lionotus												
Cyclidium sp.												
Actinophrys sol.												
Strobilidium humile												
Strombidium												
Total												
Dinoflagellata												
Rotifers and Crustaceans												

Form No. 9.

U. S. Public Health Service - Ohio River Pollution Survey

ROUTINE ANALYSES

Laboratory__________ Month of_______ 19___ Compiled by__________

Sample No.							
Stream							
Station							
Place							
Point							
Date							
Time Sampled							
River Stage-Gege Ht.(Ft.)							
River Disch.(Thous.sec.Ft.)							
WATER TEMPERATURE $^{\circ}$C.		Right					
		Center					
		Left					
		Average					
DISSOLVED OXYGEN	p.p.m.	Right					
		Center					
		Left					
		Average					
	% Sat.	Right					
		Center					
		Left					
		Average					
5 Day B.O.D. Comp. or Ave.							
COLIFORM GROUP Most Probable No. Per ml.		Right					
		Center					
		Left					
		Average					
CHEMICAL TESTS		pH					
	Parts per Million	Turbidity					
		Alkalinity					
		Acidity					
		Hardness					
		Iron					

Form No. 10.

U. S. Public Health Service - Ohio River Pollution Survey

ACID STREAM WATER ANALYSES

Stream____________________

Station____________________ Laboratory____________________

Place____________________ Compiled By:____________________

Point____________

Sample No.								Average
No. of Days Sampled								
River Disch.(Thousands of sec. ft.)								
pH								
Alkalinity (M.O.)p.p.m.								
ACIDITY	As $CaCO_3$ p.p.m.	Phenolphthalein Hot						
		Phenolphthalein Cold						
		Methyl Red						
IRON (ppm)	TOTAL							
	Ferrous	Initial						
		Final						
		Loss during Incubation						
5 Day B.O.D. (ppm)	Acid Sample	Observed						
		Oxygen equiv. of loss in ferrous iron						
		Corrected B.O.D.						
	Neutral. Sample	Observed						
		Notes						

Remarks:

Form No. 11.

U. S. Public Health Service - Ohio River Pollution Survey

RIVER MUD ANALYSES

Sample No.						
Source						
River Stage						
Date Collected						
Date Received						
Condition						
Odor						
Appearance						
Per Cent Grit (By Volume)						
Per Cent Moisture						
Volatile Matter p.p.m. (Dry Basis)						
Organic Nitrogen p.p.m. (Dry Basis)						
Oxygen Consumed p.p.m. (Dry Basis)						
Apparent Immediate Demand (p.p.m.) (Dry Basis)						
B. O. D. p.p.m. (Dry Basis)	1 day					
	3 days					
	5 "					
	7 "					
	9 "					
Value of K						

RESULTS BELOW EXPRESSED AS ORGANIC NITROGEN, OXYGEN CONSUMED, 5-DAY B.O.D., PER PPM OF VOLATILE MATTER

Organic Nitrogen					
Oxygen Consumed					
5-Day B. O. D.					

Remarks:

U. S. Public Health Service - Ohio River Pollution Survey

Form No. 12.

WORK SUMMARY - AVERAGE RESULTS

Laboratory____________________ Month of________________ 19_______ Compiled by ____________________

STATION	DAYS SAMPLED	RIVER STAGE Gage Height in Feet	RIVER DISCHARGE Thousand Sec.Ft.	WATER TEMPERATURE °C.				DISSOLVED OXYGEN								5-DAY B.O.D. p.p.m.	AGAR COUNT Per ml 24 Hrs. 37°C.	COLI-AEROGENES GROUP		CHEMICAL DETERMINATIONS					
								Parts Per Million				Per Cent Saturation								pH	Results in p.p.m.				
				R.	C.	L.	Ave.	R.	C.	L.	Ave.	R.	C.	L.	Ave.			Dilution Method M.P.N./ml.	Direct Count Per ml.		Nitrites	Turbidity	Alkalinity	Suspended Solids	
																								Total	Vol.

United States Public Health Service - Ohio River Pollution Survey

Form No. 13.

WORK SUMMARY - AVERAGE ACID STREAM RESULTS

Laboratory______________ Month of____________ 19_____ Compiled by______________

STATION	DAYS SAMPLED	RIVER DISCHARGE Thousands of Second ft.	pH	ALKALINITY to Methyl Orange as $CaCO_3$ (p.p.m.)	ACIDITY as $CaCO_3$ (p.p.m.)			IRON (p.p.m.)				5 DAY B. O. D. (p.p.m.)				
					Phenolphthalien		Methyl Red	Total	Ferrous			Acid Sample			Neutralized Sample	
					Hot	Cold			Initial	Final	Loss During Inc.	Observed B. O. D.	Ox.Equiv. of loss in Ferrous Iron	Corrected B. O. D.	Observed B. O. D.	Notes

Form No. 14

TABLE........

OHIO RIVER POLLUTION SURVEY
LABORATORY DATA

SOURCE OF DATA:
Trailer Laboratory ....
Cincinnati Laboratory ....
Laboratory Boat Kiski ....

..............................WATERSHED

SUMMARY OF INDIVIDUAL RESULTS

Sampling Point	Mileage From Mouth	Date 1940	Average Discharge c. f. s.	Temp. °C.	Dissolved Oxygen		5 Day B. O. D. p. p. m.	Coliforms M. P. N. Per ml.	pH	Turbidity p. p. m.	Alkalinity p. p. m.	Hardness p. p. m.
					p. p. m.	% Sat.						

Form No. 15

TABLE.......

OHIO RIVER POLLUTION SURVEY
LABORATORY DATA

SOURCE OF DATA:
Trailer Laboratory ....
Cincinnati Laboratory ....
Laboratory Boat Kiski ....

.............................WATERSHED

SUMMARY OF AVERAGES

Sampling Point	Mileage From Mouth	Period	Number of Samples	Average Discharge c. f. s.	Temp. °C.	D. O. p. p. m.	5 Day B. O. D. p. p. m.	Coliforms M. P. N. Per ml.	pH	Turbidity p. p. m.	Alkalinity p. p. m.	Hardness p. p. m.

Form No. 16.

Table -7A ____________ River Basin

U. S. Public Health Service - Ohio River Pollution Survey

SUMMARY OF ACID STREAM RESULTS

Stream	Sampling Point	Date	No. Samples	pH	ACIDITY P.P.M.			IRON P.P.M.	
					Methyl Red	Phenolphthalein		Ferrous	Total
						Hot	Cold		